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ANNALS OF BOTANY

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The Moisture Relations of Terrestrial Algae.¹

I. Some General Observations and Experiments.

BY

F. E. FRITSCH.

With two Figures in the Text.

A. INTRODUCTORY.

IT will be a familiar fact that a number of the subaerial algae, common in the British Isles, ordinarily occupy habitats which are liable to extreme desiccation at certain seasons of the year. Such are: the species of *Pleurococcus* and *Trentepohlia* found on tree-trunks, wooden palings, &c.; *Hormidium flaccidum* and *Prasiola crispa*, frequent on relatively heavy soils; *Zygnema ericetorum* and various terrestrial Desmids, characteristically found on acid soils; and, not so widely distributed, diverse Blue-green Algae. These forms appear little affected, in nature, even by prolonged drought, during which they retain much their normal appearance. On the advent of moisture vigorous growth is promptly resumed, and at such times the alga may often spread rapidly over adjacent bare areas. Where there is little or no competition with other forms, the alga thus gradually extends its domain, although often crowded back by other vegetation better equipped for absorbing moisture from the substratum (cf. Piercy, 1917, p. 515). It is to be noted that for all these forms the milder months of the winter are essentially the periods for rapid growth and reproduction, although in a damp summer such may go on almost continuously with occasional breaks during dry weather.

The striking characteristic of the algae under discussion is the capacity of the ordinary vegetative cells, without any marked change and without special thickening of the wall, to withstand prolonged drought—a faculty otherwise possessed only by the special resting cells (akinetes, aplanospores, zygospores, oospores) formed by aquatic and semi-aquatic algae. Moreover, the change from the resting to the active conditions and vice versa can

¹ From the Botanical Department, East London College, University of London.

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ensue in a very short space of time. There are abundant data on the power of these forms to resist prolonged and intense desiccation (cf. Bristol, 1919; Gay, 1891; Petersen, 1915; Piercy, 1917; Schroeder, 1886; West and Starkey, 1915).

A peculiar feature of most, if not of all, of these algae is the presence in many, though by no means in all, of the cells of large numbers of highly refractive granules of diverse shape and size (cf. Fritsch, 1916, p. 143; Piercy, 1917); these are apparently fatty in character, though their actual chemical nature is scarcely yet established. The more liable the habitat to drought; the more marked as a general rule the granular character of the cells—at least in the forms enumerated below which have been more particularly studied. Moreover, it seems that, as a spell of drought continues, the percentage of granular cells increases up to a certain point which is apparently the result of the dying away of a considerable number of the non-granular cells (Piercy, 1917, p. 532). In view of these facts it can hardly be doubted that the development of these granules is part of the drought-resisting equipment, but the nature of the mechanism is altogether obscure at present.

The frequently observed astonishing power of these algae to withstand prolonged desiccation practically unharmed leads one to inquire (*a*) as to possible means of obtaining moisture during periods of drought, (*b*) as to the moisture-content of the alga during such periods. The experiments and observations detailed below in relation to these points were partly conducted in the open at my home in Surrey, and partly in the laboratory and greenhouse at East London College. The material employed consisted of: *Pleurococcus naegelii*, Chod., forming a practically pure, dense growth on some wooden palings just outside Dorking; the *Hormidium* (i. e. filamentous) stage of *Prasiola crispa*, which forms extensive mats on the soil beneath Plane trees at East London College; and *Zyguema ericetorum* (the ordinary terrestrial form) from Hindhead Common and the Redlands Woods in Surrey. A few observations were also made on other forms.

In all the experiments the material was sheltered both from direct sunlight and atmospheric precipitations. For observing gain and loss of moisture weighings were made at definite intervals; no attempt was made to obtain greater accuracy than the nearest milligram, since this was more than adequate for the purpose of the experiments. The materials were exposed partly in open glass Petri dishes, partly in glazed porcelain evaporating dishes, neither of which exhibit any appreciable condensation of moisture, even on a damp day. The moisture-content of the air was determined by means of a self-recording hair hygrometer, and such temperature readings as were required were furnished by a thermograph.

B. THE LOSS OF MOISTURE BY WET MATERIAL.

If wet material of any of the algae employed is allowed gradually to dry exposed to the air, but screened from sunshine and atmospheric precipitations, it loses moisture, rapidly at first and then more and more slowly, until a weight is reached which varies only about a constant value in relation to the moisture-content of the air. At this stage the algal material presents a perfectly dry appearance like that assumed in nature during periods of drought; the dark green colour of the wet alga is replaced by a dull green. In summer this dry state is reached in the space of two or three days, in winter the time interval may be considerably longer. It will be evident, therefore, that during the summer months the beneficial effects of direct wetting by rain are of but short duration.

Drying does not, however, take place at the same rate in the different algae under consideration, a fact shown by the data given in Table I. In this experiment the different materials used were first thoroughly soaked in water and then placed in funnels for some hours until the excess moisture had drained off. They were then exposed to the air in evaporating dishes in the laboratory (av. temp. 13°C.) and weighed at frequent intervals.

TABLE I.

Results of successive weighings (between 10 a.m. and midday on each day) of soaked masses of various algae, &c. The weights are given in grammes. Attainment of air-dry weight indicated by heavy type.

<i>Zygnema ericet.</i>	<i>Hormid. stage.</i>	<i>Hormid. stage.</i>	<i>Pleurococcus.</i>	<i>Pleurococcus.</i>	<i>Spirogyra.</i>	<i>Cladophora.</i> ¹	<i>Cotton-wool.</i>	<i>Cotton-wool.</i>	<i>Seal.</i>
11.973	25.592	—	9.904	—	—	—	24.369	—	—
10.788	24.164	19.626	8.631	11.411	13.266 ²	12.622 ²	22.479	24.193	33.952
9.560	22.766	18.180	7.526	9.989	12.293	10.224	20.683	21.807	31.649
7.828	20.558	16.509	6.691	8.819	10.677	7.385	18.609	17.607	29.060
5.962	18.483	14.736	6.247	7.892	9.047	3.555	16.266	15.301	26.603
4.331	16.429	12.901	6.202	7.332	7.334	2.061	13.604	10.992	24.800
2.831	13.974	10.305	6.215	7.198	4.933	0.751	9.891	5.795	24.452
2.221	12.054	8.577	6.151	7.133	3.508	0.620	7.207	3.771	24.353
2.148	9.470	6.185	6.135	7.108	1.766	0.616	3.913	2.016	24.308
2.151	7.207	4.076	6.150	7.113	0.690	0.616	2.125	1.971	24.310
2.146	4.935	2.557	6.134	7.089	0.623	0.613	2.073	1.954	24.283
2.168	3.230	2.429	6.169	7.117	0.637	0.620	2.094	1.968	24.297
2.161	2.534	2.416	6.156	7.101	0.629	0.618	2.081	1.955	24.287
2.141	2.484	2.385	6.130	7.060	0.622	0.611	2.071	1.948	24.262
—	2.494	2.396	—	—	—	—	—	—	—
2.136	2.479	2.387	6.121	7.068	0.621	0.609	2.065	1.940	24.241

¹ A certain amount of a narrow species of *Oedogonium* also present.

² Weighed on this day at 3.45 p.m.

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It will be noticed that in practically all cases drying proceeds at a considerable and fairly uniform rate until a large portion of the contained moisture has evaporated; after that the amount of moisture lost on successive days exhibits a steady decrease to zero. When the second phase sets in, the materials appear dry to the eye, although still often damp to the touch. The first phase probably corresponds to the evaporation of moisture from between the particles or threads of material, whilst in the second we are dealing with the escape of moisture from the substance of the materials themselves. Drying takes place most rapidly in *Cladophora* (8 days), *Pleurococcus* (9 days), and *Zygnema ericetorum* (10 days); next come *Spirogyra* (11 days)¹ and the cotton-wool (11 and 12 days respectively), whilst the *Hormidium* stage of *Prasiola* is the slowest of all the algae (15 and 16 days respectively). The clay soil employed had not yet dried completely at the end of the experiment (18 days). These data will be discussed more fully below (p. 14).

It will be evident from a glance at Table I that the amount of moisture lost varies appreciably in the different materials. This is no doubt due both to a varying capacity for the absorption of liquid water and, to a minor extent (cf. below), to a varying power of retention of moisture on the part of the air-dry material. The relevant data are summarized in Table II. *Pleurococcus* exhibits by far the smallest moisture-holding

TABLE II.

Comparison of the amounts of moisture held by the different materials dealt with in Table I, in relation to volume and air-dry weight.

Nature of material.	Volume of same.	Moisture lost.	Ditto per c.c. ²	Ratio of moisture lost to air-dry weight.
	c.c. ²	gm.		
<i>Zygnema ericetorum</i>	3.5	9.837	2.8	4.6
<i>Hormidium</i> stage (<i>Prasiola</i>)	6.0	23.113	3.8	9.3
" " "	5.5	17.241	3.1	7.2
<i>Pleurococcus</i>	11.0	3.783	0.3	0.6
" "	11.5	4.351	0.3	0.6
<i>Spirogyra</i>	1.5	12.645	8.4	20.3
<i>Cladophora</i>	1.5	12.013	8.0	19.7
Cotton-wool	2.0	22.304	11.1	10.8
" "	2.0	22.253	11.1	11.5
Soil	10.0	9.711	0.9	0.4

capacity (smaller even than that of the soil) among the algae studied, whilst the two freshwater algae have the largest; the same volume of cotton-wool, however, exceeds them in absorptive capacity. It is quite clear from a study of the figures in the last column of Table II that in

¹ The slower rate of drying of *Spirogyra* as compared with *Cladophora* is no doubt due to the mucilaginous character of the walls.

² These data are only approximate.

a mass of soaked freshwater alga, uncombined water makes up a far larger percentage of the total weight than it does in the case of any of the terrestrial algae studied. This implies that in proportion to the air-dry weight the terrestrial forms have a smaller water-absorbing capacity than the others. Attention may be drawn to the fact that the *Hormidium* stage of the *Prasiola* is able to take up most moisture among the terrestrial forms investigated.

C. THE RELATION TO AIR HUMIDITY.

During August and September of 1919 a large number of weighings were made of algae, mosses, and lichens, growing on different substrata, with the object of ascertaining the extent to which their moisture-content varied with that of the air; the materials were in all cases allowed to attain an air-dry condition before any weighings were undertaken. The method of experimentation proved unsuitable for detailed work, but a few of the general results are summarized in Table III in order to give some idea of the values involved. It will be noticed that there is in all cases

TABLE III.

Range of moisture-content of terrestrial Cryptogams and various substrata for period of experiment (4-6 weeks), August-September, 1919.

Nature of material.	Approx. area.	Minimum weight.	Maximum weight.	Range.
	sq. cm.	gm.	gm.	gm.
Dense covering of <i>Pleurococcus</i> } on thin layer of soil	38.5	5.686	5.852	0.166
Holly bark with covering of } <i>Pleurococcus</i>	46.8	4.753	4.908	0.155
Holly bark alone	44.5	1.574	1.670	0.096
Pinus bark with <i>Parmelia phy-</i> } <i>sodes</i>	large	6.118	6.640	0.522
Pinus bark alone	42.8	6.040	6.471	0.431
Pinus bark with dense <i>Pleuro-</i> } <i>coccus</i>	35.1	4.711	5.082	0.371
Moss ¹	large	1.600	1.822	0.222
Powdered soil	78.5	18.141	18.384	0.243
Pure stratum of <i>Pleurococcus</i>	78.5	2.003	2.167	0.164

a considerable range in moisture-content which is particularly striking in relation to the total weight in the case of the moss and *Pleurococcus*. The various substrata, however, also take up a considerable amount of moisture from the air, and there would appear to be differences in this respect between the bark of different trees. I hope to deal more fully with this aspect of the matter in a later communication.

At the time at which these experiments were undertaken, the maximum moisture-content of the air (usually 75-85 per cent. of saturation) was

¹ *Hypnum cupressiforme*, L., var. *filiforme*, Brid.

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generally attained about 8 a.m.¹; this was followed by a rapid drop to between 40 and 50 per cent., a point reached between 2 and 4 p.m. For some two or three hours the air remained in this condition, but usually about 5 or 6 p.m. a rise of moisture-content took place, and this increase continued steadily till the maximum was reached about 8 a.m. the next morning. On damp or rainy days the pen of the hygrograph traced out an almost straight line, usually ranging about 80 per cent. In all probability these data are typical of the south of England for this time of the year. A similar sequence seems frequent in winter, the maximum humidity being attained soon after daybreak, but the rise in the afternoon commences almost immediately after the minimum is reached.

With the falling humidity, soon after 8 a.m.,¹ all the various materials investigated give off moisture rapidly, but this is arrested soon after midday and for part of the afternoon the weight often remains almost constant. Soon after 5 p.m., however, an increase is observed, and this continues steadily until dusk. No further weighings were made after that time, but there is evidently a very considerable absorption of moisture overnight, since the weight at dusk is always far less than that recorded in the early morning (cf. Table V). When the middle of the day is dry as compared with the morning, a considerable daily range is observed, whilst when the humidity of the air remains high the daily range is small. There may thus be a considerable diurnal range in moisture-content, but losses sustained on a dry day are often more than made good overnight. When the humidity of the air remains high for several days in succession, a steady increase in weight may be observed day after day, implying a very marked absorption of moisture (cf. Table IV).

TABLE IV.

Comparison of weights of diverse materials in the early morning of successive days (increase indicated by +, decrease by —; in all cases the increments are expressed as percentages of the total range observed during the period of the experiment²).

Date.	<i>Pleurococcus</i> on thin layer of soil.	Soil with moss pro- tonema and <i>Hormidium</i> .	Holly bark with <i>Pleurococcus</i> .	<i>Pinus</i> bark with ditto.	Remarks.
Aug. 16	— 2	— 4 $\frac{1}{2}$	— 3 $\frac{1}{2}$	— 1 $\frac{1}{2}$	Hygrograph curve normal.
" 17	+ 12 $\frac{3}{4}$	+ 8 $\frac{3}{4}$	+ 22 $\frac{3}{4}$	+ 3 $\frac{3}{4}$	
" 18	— 15 $\frac{1}{2}$	— 3	+ 2 $\frac{1}{2}$	+ 7 $\frac{1}{2}$	
" 19	+ 5 $\frac{1}{2}$	+ 30 $\frac{1}{2}$	+ 32 $\frac{1}{2}$	+ 32 $\frac{1}{2}$	Hygrometer not below 75 from 8 p.m. on 18th till 10 a.m. on 21st.
" 20	+ 11 $\frac{5}{8}$	+ 16 $\frac{5}{8}$	+ 49	+ 11	
" 21	— 3 $\frac{5}{8}$	+ 4 $\frac{5}{8}$	— 41 $\frac{1}{2}$	+ 3 $\frac{5}{8}$	
" 22	— 24 $\frac{5}{8}$	— 44 $\frac{1}{2}$	— 19	— 30	Hygrometer fell to 41 at 4 p.m. on 21st.

¹ Summer time, i.e. one hour earlier than G.M.T.

² This mode of expression appears the most suitable means of indicating the amount of absorption in relation to the total absorptive capacity.

. Material kept in a constantly saturated atmosphere exhibits an increase in weight above that normally observed in the open air, and, conversely, material placed in a desiccator over calcium chloride exhibits a greater decrease in weight than is to be observed in the open. In either case, however, an approximately constant value is reached after some days. If such material is subsequently left exposed to the air, it exhibits a decrease and increase in weight respectively till that normal for the hygrometric state of the atmosphere is attained.

In relation to the absorption of atmospheric moisture by various fabrics, Trouton and Pool (1906), as well as Masson and Richards (1906), have

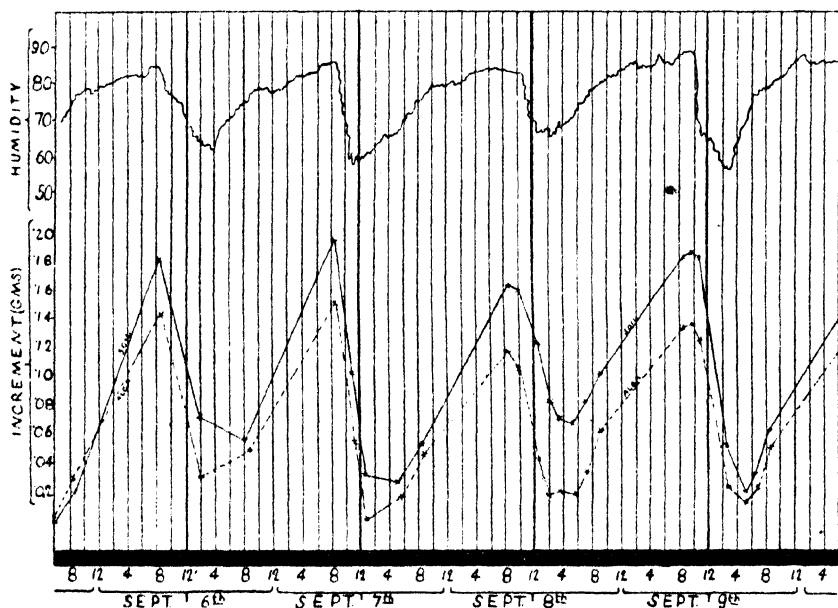


FIG. 1. Comparison of change in moisture-content of the same area of powdered soil and *Fleurococcus naegelii* in relation to atmospheric humidity. The hygrograph record for the period Sept. 6-9, 1919, is reproduced at the top, the graphs of moisture-content of soil (continuous line) and alga (interrupted line) below. Noon of each day is indicated by a heavier line.

independently established that (in the words of the former) 'the weight of water absorbed or held by a given material under different conditions of moisture and temperature of the atmosphere appeared to depend only on the hygrometric state (i.e. the ratio of actual vapour pressure to the maximum possible), though of course the actual amount of moisture present in the atmosphere for the same ratio is very different at different temperatures' (loc. cit., p. 292). In my experiments the materials used were scarcely ever allowed to attain an equilibrium value, and consequently I am at present unable to say whether this statement holds true for the algae investigated. If it obtained, the range (over a period of several

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weeks) in winter should be approximately the same as in summer, since the range in percentage humidity of the air is about the same, but the data at present obtained indicate a smaller range in winter than in summer. On the other hand, the second relation recorded by Trouton and Pool was frequently observed, viz. that there is a much greater reduction in weight for a given change in vapour pressure when near saturation than is subsequently obtained.

The phenomena referred to in the preceding paragraphs of this section are of course to be observed in all hygroscopic materials and are a result of adsorption and imbibition. In the case of material composed of living plant-cells, however, it would seem probable that moisture imbibed by the cell-wall would tend to become absorbed by the protoplast (cf. below, p. 13), so that moisture would be taken up more rapidly than in the case of inanimate material. Indeed, if one compares living with dead material a difference is very obvious (cf. Table V and Fig. 1). It will be noticed that the alga corresponds much more markedly with changes in the

TABLE V.

Comparison of change in moisture-content of the same area of powdered soil and *Pleurococcus naegelii* in relation to atmospheric humidity (increase indicated by +, decrease by —; in all cases the increments are expressed in percentages of the total range observed during the period of the experiment, cf. foot-note on p. 6).

Date and hour. ¹	Soil.	<i>Pleurococcus.</i>	Hygrometer reading.
	Total range : 0.243 grm.	Total range : 0.164 grm.	
Sept. 6, 8.15 a.m.	+69 $\frac{1}{8}$	+70 $\frac{1}{8}$	84.5
„ 6, 2 p.m.	—46 $\frac{7}{8}$	—69 $\frac{1}{8}$	68
„ 6, 8.15 p.m.	—6 $\frac{1}{8}$	+11	73
„ 7, 8.20 a.m.	+54	+62 $\frac{7}{8}$	84.5
„ 7, 11 a.m.	—37 $\frac{7}{8}$	—61	70
„ 7, 12.30 p.m.	—31 $\frac{1}{8}$	—39	58
„ 7, 5.15 p.m.	—1 $\frac{1}{8}$	+10 $\frac{3}{8}$	65
„ 7, 8.50 p.m.	+12	+17	74
„ 8, 8.20 a.m.	+47 $\frac{1}{2}$	+47	82
„ 8, 10 a.m.	—2 $\frac{3}{8}$	—8	82
„ 8, 12.25 p.m.	—26 $\frac{3}{8}$	—39	67
„ 8, 2 p.m.	—17 $\frac{1}{2}$	—14 $\frac{3}{8}$	66
„ 8, 3.30 p.m.	—4 $\frac{1}{2}$	+ $\frac{3}{8}$	66
„ 8, 5 p.m.	— $\frac{3}{8}$	— $\frac{3}{8}$	66
„ 8, 7.30 p.m.	+5	+9 $\frac{3}{8}$	71
„ 8, 8.55 p.m.	+9	+17	75.5
„ 9, 8.15 a.m.	+33 $\frac{3}{8}$	+42	85.5
„ 9, 9.30 a.m.	+2	+1 $\frac{1}{2}$	86.5
„ 9, 10.45 a.m.	— $\frac{3}{8}$	—6 $\frac{3}{8}$	84
„ 9, 2.5 p.m.	—55 $\frac{1}{8}$	—66 $\frac{1}{8}$	63
„ 9, 5.25 p.m.	—15 $\frac{3}{8}$	—9 $\frac{3}{8}$	56
„ 9, 6.25 p.m.	+5 $\frac{3}{8}$	+6 $\frac{1}{2}$	64
„ 9, 8.15 p.m.	+13 $\frac{3}{8}$	+18 $\frac{1}{2}$	75
„ 10, 8.15 a.m.	+54	+68 $\frac{1}{2}$	86

¹ Summer time.

humidity of the air than does the soil (cf. especially the afternoon of each day). The alga also absorbs in general a greater percentage of the possible amount of moisture when the air is damp and parts with it more rapidly as the humidity of the air falls (Table V). Moreover, the rate of loss in the morning falls off more rapidly in the case of the alga than it does in the case of the soil. There is some evidence, therefore, to indicate that the alga constitutes a transpiring mechanism which in dry air rapidly parts with moisture, if it has been previously absorbed in quantity, until an osmotic check comes into play due to increasing concentration of the sap. It is no doubt the concentrated sap that is in great part responsible for the rapid uptake of moisture when the humidity of the air again increases.

Similar results were obtained by comparing a mass of air-dry *Cladophora* with soil. It seems that, if different kinds of materials are compared in this way, there is great correspondence in the variations in moisture-content for small differences in atmospheric humidity, but that with larger differences the correspondence is not so marked.

In the experiments referred to in this section, as already stated, the materials were placed under shelter. In a more exposed position, especially if reached at certain hours of the day by the sun, the diurnal range would of course be greater and the absorption of moisture from dew on many a night appreciable.

D. THE CONDITION DURING DROUGHT.

The data given in the preceding section are in large part a natural outcome of the hygroscopic character of the materials employed, but I may be pardoned for dealing with them in some detail, since in relation to these lowly terrestrial plants they may be of considerable significance. If a mass of *Pleurococcus*, weighing 2.003 grm. and with a superficial area of about 78.5 square centimetres, can, under the conditions of the experiment, increase in weight by 0.164 grm. (cf. Table III), i. e. by an amount rather over 8 per cent., this must mean the intake of a considerable quantity of atmospheric moisture. This, being retained for a number of hours at least, may be sufficient to admit of a certain amount of growth. It is to be noticed that the maximum moisture-content of the air and of the alga was realized at about 8 a.m. summer-time (p. 6), i. e. some hours after daybreak, so that a certain amount of photosynthesis would be possible, if the quantity of moisture present were adequate. It is not unlikely, moreover, that the above-mentioned granules constitute a form of food-reserve (cf. Piercy, 1917, p. 533), laid up during periods of active metabolism, to be used for growth overnight at times of high moisture-content during subsequent dry periods.

The question arises whether moisture adsorbed on the surface and imbibed into the cell-wall is able to obtain entry into the protoplast, a point

which is very difficult to settle directly. A number of facts, however, point to this being the case, amongst others those mentioned above (p. 9).

When cells of *Pleurococcus naegelii* are mounted in water and examined under the microscope, they appear more or less rounded off (Fig. 2, *a*). The cell-wall is distinguishable into two regions, a denser outer portion and a less dense, presumably mucilaginous, inner portion which appears as a slight space separating the protoplast from the outer layer of the membrane (Fig. 2, *a*, and Chodat, 1902, Fig. 195, 196). If such cells be allowed to dry gradually on the slide, they retain their normal appearance until air-films commence to arise around them. At this stage the cells

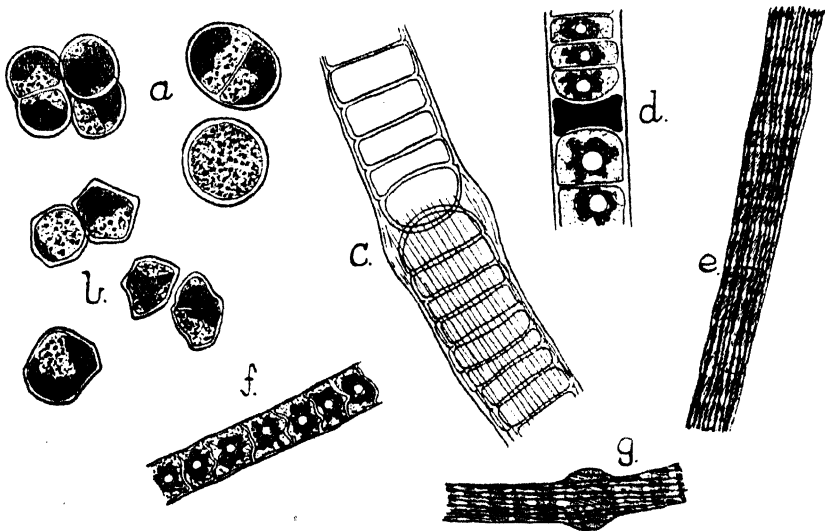


FIG. 2. *a-b*, *Pleurococcus naegelii*. *a*, cells mounted in water; *b*, 'dry' cells, mounted in cedar-wood oil. *c-g*, *Hormidium* stage of *Prasiola crispa*. *c*, portion of a thread, on part of which the longitudinal striation is shown; *d*, part of a thread with a 'concave cell'; *e*, 'dry' thread (cell-contents shaded); *f*, ditto, mounted in cedar-wood oil; *g*, 'dry thread', showing dilation due to a pair of 'concave cells'. (All figures $\times 800$.)

become angular in outline (Fig. 2, *b*), but there is no considerable contraction, though the mucilage layer of the wall appears thinner in such dry cells.¹ No further change can be detected, even in cells unsupplied with liquid moisture for several days. There is no marked contraction of the protoplast, which appears to be as closely invested by the cell-wall as in the wet cell (cf. below).²

In the case of the *Hormidium* stage of *Prasiola crispa* the effects of drying are slightly different. Even in the wet condition the filaments of this alga exhibit a delicate longitudinal striation of the cell-walls which

¹ Examined in cedar-wood oil.

² The slight contraction of the cells of *Pleurococcus* on drying appears of advantage in connexion with the vertical habitat frequented by this alga. It is difficult to conceive how adhesion to the substratum could be maintained if the cells were subject to frequent violent contractions.

generally has a slight spiral trend (Fig. 2, *c*). As drying proceeds the striations become somewhat more pronounced. When air-films appear around the filaments, the latter exhibit a sudden, rather marked contraction in width, and at the same time the striations become very prominent as a number of deep furrows and ridges (Fig. 2, *e*). The transverse contraction of the threads amounts usually to one-fifth or one-quarter of the original width, though now and again considerably greater (up to as much as five-twelfths); there is no appreciable longitudinal contraction, and what there is seems to set in only after some time. It appears that the delicate longitudinal striae seen on the walls of the wet filaments are lines of weakness along which the membrane becomes gradually folded during drying, so that the cell-wall shrinks round the contracting protoplast and remains closely investing it. In dry threads mounted in cedar-wood oil no space is visible between the thin wall and the protoplast (Fig. 2, *f*). According to Imhäuser (1889, p. 5) the inner parts of the membrane are mucilaginous, a conclusion at which I have also arrived. No doubt, therefore, there is a certain contraction of the cell-wall on drying, but there is also a decrease in the transverse dimensions of the protoplast to the extent of about one-quarter of its previous width, as shown by measurements of the same thread in the wet and dry conditions.

It is noticeable that the septa do not stand out in the dry threads, but become more or less arched or zigzagged (Fig. 2, *f*), a condition frequently retained to some extent even in the wet thread, especially if it be of relatively considerable width. The characteristic striation of the longitudinal walls just described has been observed in filaments of this alga from diverse terrestrial substrata in the south of England. On the other hand, it is not referred to by Imhäuser (*loc. cit.*), nor have I been able to discern it in the abundant Antarctic material of this species which I have had the opportunity of examining. It may thus be a special characteristic of the extreme terrestrial form.

The dry threads of the *Hormidium* stage always show a number of prominent knob-like swellings (Fig. 2, *g*), not seen in the wet threads and marking points at which the filament has scarcely contracted beyond its original dimensions. These are the places at which the characteristic 'concave cells' (plano-concave when in pairs) occur which thus resist contraction on drying to a much more marked extent than the other cells of the threads. The 'concave cells' (Fig. 2, *d*) are always either moribund or dead; at first they are a deep homogeneous green in contrast to the lighter green of the ordinary cells; but later on the pigment disappears and the cells appear colourless. A chloroplast with pyrenoid is still often demonstrable in these cells before they lose their pigment. The concavity of the septa adjoining living cells is probably due to the latter pushing their way into these cells by virtue of their turgescence (*cf.* Imhäuser, 1889,

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p. 5). The cause of the non-contraction of the 'concave cells' is still obscure; it may either be due to denser contents or to a firmer wall. It is possible that one of their functions is to prevent transverse contraction of the threads exceeding a certain limit. At the same time it is probable that the cell-membrane at these points of inflation will be in a state of tension during drought which may produce a weakening tending to subsequent rupture at these places. Certain it is that the frequent fragmentation of the threads of this alga not uncommonly takes place at the points where such 'concave cells' occur.

It is quite likely that the 'concave cells' are non-granular ones which die away owing to their incapacity to resist drought. It is noticeable that such cells are often far more abundant in non-granular than in granular threads, although this is not an invariable rule. In material of the same alga growing on sandy soil in the Farnham Lane at Haslemere, a more rigorous habitat than that at East London College, these cells were very plentiful in the threads.

The behaviour of the threads of *Zygnema ericetorum* on drying is again different to that of the other two terrestrial forms studied. As in the case of the *Hormidium* stage of *Prasiola* transverse contraction takes place suddenly when the air-films appear around the threads, but this is not associated with longitudinal folding of the wall along definite lines of weakness, although irregular longitudinal folds are often observed. Both cell-wall and protoplast shrink, but in the case of the latter this takes place to a varying extent. In some threads most or all of the cells show little transverse contraction of the protoplast, which remains in close contact with the shrunken cell-wall. In others there is pronounced shrinkage of the protoplast, which then becomes separated from the membrane along the sides of the cell. In such cells the septa usually remain more or less fully stretched, whilst the longitudinal walls cave inwards to some extent, so that each cell comes to have somewhat of the shape of an hour-glass. This is still more pronouncedly seen in the protoplast, which remains in firm contact with the entire width of the septum at either end of the cell, whilst the middle part is often very pronouncedly constricted.

It is worthy of note that the tendency exhibited by these terrestrial algae for the membrane on drying to remain in contact with the protoplast is also observed in some aquatic forms of this group. In *Spirogyra* and *Closterium acerosum* the cell-contents usually contract markedly away from the membrane on drying, while a narrow species of *Oedogonium* showed changes similar to those just described for *Zygnema ericetorum*. In the case of *Cladophora* the young green branches generally exhibit marked plasmolysis on drying, but in the case of the thick-walled dark-green winter stages with dense contents the cell-wall remains closely investing the protoplast. This fact is significant in view of the dominance of

Cladophoraceae on certain South African marshes which are dry land for about half the year.

The usually close contact between the membrane and the protoplast in the cells of the above-discussed terrestrial algae during drought renders it highly probable that atmospheric moisture imbibed into the cell-wall will pass readily into the adjacent protoplast. And it is scarcely likely that the latter will not profit by any atmospheric moisture thus absorbed, even if the amount is not nearly sufficient to distend the cell to its normal 'wet' dimensions. Even should a very slight space exist between the protoplast and the shrunken membrane, the fact that the inner parts of the latter are mucilaginous will involve their swelling and the filling out of this space as soon as any appreciable quantity of moisture has been absorbed. The least well-equipped form as regards maintenance of contact between wall and protoplast is the *Zygnema*, which, however, has thicker mucilaginous walls than the other two algae.

In this connexion attention may be drawn to the fact that liquid water is absorbed rapidly by the dry mats both of *Zygnema ericetorum* (Fritsch, 1916, p. 139) and of the *Hormidium* stage of *Prasiola*. In the former case this is due to the abundant mucilage, in the latter the longitudinal folds probably act as capillary channels for the absorption of liquid water. In this way, in the case of such soil-inhabiting algae, a certain amount of moisture may possibly also be absorbed with the help of threads penetrating into the substratum. Masses of dry *Pleurococcus*, on the other hand, are not easily wetted, the difficulty lying in the displacement of the air-films around the cell-groups. In view of the slight contraction exhibited by the protoplasts of this alga in drying, however, very small amounts of moisture are probably adequate to start the full vital machinery.

It is thus rendered probable that moisture imbibed from the air by the cell-walls of these algae reaches the protoplast, and that at times when the air remains near saturation point for long periods sufficient moisture may thus be absorbed to admit of a certain amount of growth taking place. Attempts to establish the occurrence of such growth by direct measurement of material kept in a saturated atmosphere have, however, so far proved quite inconclusive.

During prolonged drought, with a low percentage of air humidity, the protoplast will part with moisture to the adjacent membrane and the latter will part with it to the air until the sap in the cells of the alga reaches such a concentration that the imbibition forces in the membrane are balanced by the osmotic suction of the sap. When this condition of equilibrium is reached the algal material will neither gain nor lose moisture and will exhibit a constant average weight. That this stage is attained soon after drying sets in has been shown in Section B, and it remains now to consider the condition of the protoplast at this time.

Neither in the cells of *Pleurococcus naegelii* nor in those of the threads of the *Hormidium* stage of *Prasiola crispa* are large vacuoles distinguishable. For the latter alga this fact has already been established by Imhäuser (1889, p. 5), whilst none of the published figures of *Pleurococcus* show such vacuoles (cf. Chodat, 1902, pp. 279–83; Gay, 1891, Pl. XIV, Figs. 136, 137; Nägeli, 1849, Tab. IV, E, Fig. 2; Snow, 1899, Pl. XI). Their absence is also evidenced by the behaviour of these two algae when immersed in strong solutions of sea-salt. Thus the protoplast of *Pleurococcus* exhibits no evident contraction when the cells are mounted in a 10 per cent. solution of Tidman's sea-salt, so that the amount of water which such a solution (with an osmotic pressure of some seventy-five atmospheres) can withdraw from the protoplast is negligible. There are two possible explanations: either the quantity of moisture present in the cells is very small or the sap which is dispersed through the cytoplasm has so high a concentration as to exceed that of the strong solution employed.

The cells of the *Hormidium* stage are markedly plasmolysed by a 10 per cent., very slightly by a 5 per cent., solution of sea-salt; a 4 per cent. solution (osmotic pressure about thirty atmospheres) has no effect. It is noticeable that the protoplasts of most of the cells do not show any very considerable contraction, even in the strongest solution employed. It appears therefore that, by contrast with *Pleurococcus*, the cells contain more moisture and the sap is not as concentrated, but the amount is small as compared with a freshwater alga.

In the case of *Zygnema ericetorum* some of the cells show obvious vacuoles, whilst in others the cytoplasm fills practically the whole cavity¹ (West and Starkey, 1915, cf. Fig. 1 and 2 with Fig. 4). Immersion in a 10 per cent. solution of sea-salt causes pronounced plasmolysis of all the cells, whilst a 3 per cent. solution only produces slight plasmolysis and is evidently not much stronger than the cell-sap. It is noticeable that the degree of plasmolysis varies greatly in different cells and different filaments,² which accords with the varying amount of contraction noted on drying.

It will be realized that these facts agree well with the observed behaviour of the different algae during drying. As regards the influence of the different strengths of plasmolysing solution used, the three algae can be placed in the order: *Pleurococcus*, *Prasiola*, *Zygnema*. The same order expresses the relative degree of contraction during drying. If no appreciable amount of liquid can be abstracted from the cells of *Pleurococcus* by a 10 per cent. sea-salt solution, then it is hardly likely that there will be any sensible loss of moisture from them on exposure to dry air. On the other

¹ In the extreme terrestrial form found at Hindhead (Fritsch, 1916) vacuoles were never observed in the cells.

² It is probable that *Hormidium flaccidum* would show similar relations. Accurate determinations of osmotic pressures were felt to be unnecessary in the present connexion, the more so as it is proposed to deal with this aspect of the matter in a subsequent communication.

hand, *Zygnema* and the *Hormidium* stage evidently part with moisture more readily, although the thick mucilaginous walls in the case of the former will oppose evaporation, and the process may be expected to stop sooner than in the *Hormidium*, in spite of the usually larger quantity of sap present. We thus arrive at an explanation of the relative rates of drying of these different algae noted on p. 4. Moreover, seeing that *Pleurococcus* exhibits little contraction on drying, it is not to be expected that it will have any great absorptive capacity for moisture, which should be much less than in the case of the *Hormidium* stage or the *Zygnema* (cf. Table II).

The results above obtained also indicate the probability that the cells of these terrestrial algae still hold a larger or smaller amount of moisture in the air-dry condition. This problem can be attacked by determining the loss of moisture when such air-dry material is subjected to prolonged heating at temperatures sufficiently high to kill the protoplasts and to drive off the uncombined water.

E. THE MOISTURE-CONTENT OF THE AIR-DRY ALGA.

A considerable number of determinations of this kind were made with the *Hormidium*-stage of *Prasiola*, of which abundant material was available. In all cases the pieces of alga-mat were allowed to reach an air-dry condition in the laboratory, and were then subjected to a temperature slightly below 100° C. for several hours until a constant weight was attained. After the dry weight had thus been determined the material was allowed to stand exposed to the air of the laboratory for several days in order to establish what percentage of the moisture lost could be reabsorbed from the atmosphere. Some of these results are given in Table VI.

TABLE VI.

Determination of dry weight and of amount of moisture permanently lost after heating in the case of the *Hormidium* stage of *Prasiola crispa*.

	<i>Air-dry weight of alga.</i>	<i>Dry weight of same.</i>	<i>Moisture lost.</i>	<i>Ditto in % of dry weight.</i>	<i>Weight after exposure to air.</i>	<i>Permanent loss.</i>	<i>Ditto in % of total loss.</i>
	gram.	gram.	gram.		gram.	gram.	
I.	1.096	1.009	0.087	8.6	1.066	0.030	34.5
II.	1.170	1.076	0.094	8.7	1.140	0.030	31.9
III.	1.806	1.746	0.150	8.6	1.825	0.071	47.3
IV.	3.616	3.420	0.196	5.7	3.520	0.096	49.0
V.	2.479	2.234	0.245	10.9	2.422	0.057	23.3
VI.	2.385	2.190	0.195	8.9	2.336	0.049	25.1

The alga in all cases loses a considerable amount of moisture on heating, usually between 8 and 9 per cent. of the dry weight. On subsequent exposure to air a part of this moisture is regained, but a quarter to

one-half of it is permanently lost, and it is probably justifiable to assume that some of this at least represents moisture that was retained by the living protoplasts and is incapable of being reabsorbed by them when dead. The considerable range exhibited by the figures in the last column of Table VI is probably in part due to the varying purity of the material; it is very difficult to get rid of all soil and other intermingled matter. Moreover, the air-dry weight depends on the hygrometric state of the air on the day of the experiment. A range between 25 and 35 is probably the normal for this alga.

In Table VII the results of similar experiments with air-dry material of the different forms discussed in this paper are given for comparison with one another and with freshwater algae, cotton-wool, and soil. The materials are the same as those dealt with in Tables I and II.

TABLE VII.

Determination of dry weight and of amount of moisture permanently lost on heating of various materials.

<i>Nature of material.</i>	<i>Air-dry weight of same.</i>	<i>Dry weight of same.</i>	<i>Moisture lost.</i>	<i>Ditto in % of dry weight.</i>	<i>Weight after exposure to air.</i>	<i>Permanent loss.</i>	<i>Ditto in % of total loss.</i>
	gram.	gram.	gram.		gram.	gram.	
<i>Zygnema</i>	2.136	1.942	0.194	9.9	2.112	0.024	12.4
<i>Hormidium</i>	2.479	2.234	0.245	10.9	2.422	0.057	23.3
"	2.385	2.190	0.195	8.9	2.336	0.049	25.1
<i>Pleurococcus</i>	6.121	5.753	0.368	6.4	5.989	0.132	35.9
"	7.060	6.737	0.323	4.8	6.959	0.101	31.3
<i>Spirogyra</i>	0.620	0.553	0.067	12.1	0.618	0.002	3.0
<i>Cladophora</i> ¹	0.613	0.540	0.073	13.5	0.607	0.006	8.2
Cotton-wool	2.065	1.900	0.165	8.7	2.041	0.024	14.5
" "	1.941	1.811	0.130	7.2	1.932	0.009	7.0
Soil	24.241	23.702	0.539	2.3	24.054	0.187	34.7

On the whole these results are quite in harmony with the conclusions arrived at in the preceding sections. It is to be noticed that, by comparison with its dry weight, *Pleurococcus* exhibits the lowest percentage loss of moisture on heating of all the algal forms studied, so that it seems probable that the amount of moisture held by the air-dry cells of this alga is small (cf. above, p. 14). On the other hand, *Pleurococcus* shows the highest permanent loss (viz. 35.9 and 31.3 per cent. of the total) among the algae investigated in this experiment, although the *Hormidium* stage may exhibit a similar high value (cf. Table VI). It is legitimate to assume that most, at least, of the permanent loss observed in the case of *Pleurococcus* is due to inability of the dead protoplasts to take up the moisture formerly held; in other words, that relative to the total amount of moisture retained by the air-dry alga that contained within the protoplast constitutes a large

¹ A certain amount of a narrow species of *Oedogonium* also present.

amount. Support is thus given to the conclusion that in the air-dry condition the protoplasts of *Pleurococcus* still retain a relatively large percentage of the moisture that this alga can absorb, although the total amount held is smaller than that observed in the other forms. This probably in great part accounts for the lack of any marked contraction of the cells of this alga on drying.

Zygnema ericetorum and the *Hormidium* stage of *Prasiola* show about the same percentage loss of moisture on heating the air-dry material, the variation noted in the case of the latter (cf. also Table VI) possibly according with the varying degree of contraction exhibited by the threads (p. 11) on exposure to drought. But these two algae differ altogether in the percentage of moisture that is permanently lost after heating, the amount being much smaller in the case of *Zygnema*. This may be taken to mean that a smaller quantity of moisture is retained within the protoplasts of this alga than in those of the *Hormidium* stage, which is again in agreement with the fact that those of *Zygnema* show a much more marked contraction on drying than do those of the other alga. The fact that, nevertheless, the total amount of moisture lost by *Zygnema* on heating is about the same as that lost by the *Hormidium* stage is no doubt due to the high mucilage-content of the walls of the former.

By contrast with these terrestrial algae the two aquatic forms studied give quite different results. In the first place, the quantity of moisture lost on heating is higher in proportion to the dry weight, which is probably due to the water-retentive power of the mucilage of the *Spirogyra* and the thick membranes of the *Cladophora*. In the second place, the percentage of permanent loss to total loss is low, even very low in *Spirogyra*, indicating that the air-dry alga contains only very small amounts of moisture that cannot subsequently be reabsorbed from the air. In this respect the difference as compared with the *Hormidium* stage and with *Pleurococcus* is very striking. It is noticeable that the amount of permanent loss is greater for *Cladophora* than for *Spirogyra* (the difference would perhaps be even more pronounced if *Oedogonium* had not been intermixed with the *Cladophora*), and in this connexion the fact that a great part of the *Cladophora* was in the winter condition should be noted, since such threads (cf. above) exhibit less contraction on drying and presumably retain some moisture in their protoplasts.

Comparison of cotton-wool with the algae investigated again shows a relatively low percentage of permanent loss, although the results are somewhat variable and not altogether clear. The fact that there is nevertheless an appreciable permanent loss in the case of the cotton-wool indicates some alteration of the fibres as a result of heating, whereby their absorptive capacities are reduced. The possibility of such alteration in the case of the algae experimented upon may not be overlooked, but the

results obtained with freshwater algae, where the membranes are presumably of much the same nature as in the terrestrial forms, indicate that here no great change of this kind can obtain. The appreciable permanent loss shown by the clay soil is probably the result of alteration of the imbibitional powers of some of the soil constituents.

Examination of cells of *Pleurococcus* which have been subjected to such prolonged heating shows that the protoplasts in all of the cells have contracted away from the wall, presenting a very different appearance to that of the air-dry alga. The degree of contraction, however, varies considerably, and appears in general to be least in cells with plenty of granules. This observation supports the view that a considerable amount of moisture has been given up from the protoplasts. In the case of the heated threads of the *Hormidium* stage contraction of the protoplasts is likewise observed, but it is scarcely as pronounced as in *Pleurococcus*, implying a smaller moisture-content in the air-dry condition. In both cases, however, the protoplasts still fill a large part of the cell-cavity and are not as strongly contracted as in most plant-cells after heating. The cells in the heated threads of the *Zygnema* do not differ much in appearance from the air-dry ones, although contraction of the protoplasts is still more pronounced.

F. SUMMARY AND GENERAL CONCLUSIONS.

The following are the principal conclusions that emerge from the foregoing considerations:

1. The protoplasts of the various terrestrial algae examined exhibit either a complete absence (*Pleurococcus*, *Hormidium* stage of *Prasiola*), or a paucity (*Zygnema ericetorum*), of large vacuoles, most of the sap apparently being dispersed through the cytoplasm.

2. In the air-dry condition a considerable proportion of this sap is retained, more in the case of *Hormidium* and *Pleurococcus* than in the case of *Zygnema*.

3. When drying takes place contraction occurs in such a way that the cell-wall remains closely investing the protoplast (*Pleurococcus*, *Hormidium* stage) or in contact with it at certain points (*Zygnema*).

4. Moisture imbibed into the walls from the atmosphere will therefore reach the protoplast, especially in the case of *Pleurococcus* and the *Hormidium* stage of *Prasiola*.

5. The cells of *Pleurococcus* exhibit no appreciable amount of contraction on drying, whilst in the *Hormidium* stage of *Prasiola* there is evident contraction accompanied by longitudinal folding of the walls along special lines of weakness. In *Zygnema* the amount of contraction is variable. The capacity for absorbing moisture is least in *Pleurococcus* and greatest

in the *Hormidium* stage of *Prasiola*, but in all the terrestrial algae investigated is far smaller than in aquatic Algae.

6. Terrestrial algae, therefore, and especially *Pleurococcus*, require only relatively small amounts of moisture to replace that lost by the protoplast in drying.

7. Appreciable amounts of moisture can be absorbed from the atmosphere at times when the humidity of the air is great, and it is probable that a certain amount of growth can be effected during such periods.

8. Terrestrial algae respond more rapidly to changes in the hygro-metric state of the air than does inanimate material. At times of low humidity they exhibit a more rapid falling off in the rate of loss of moisture than non-living material.

9. The sap of the terrestrial algae investigated shows a high degree of concentration, which probably becomes so marked after a little evaporation has taken place that further loss of moisture is prevented. The rate of drying of the different forms, however, differs considerably, the *Hormidium* stage of *Prasiola* taking longest to attain an air-dry condition.

It thus appears that these terrestrial algae are well equipped for the vicissitudes which they have to face. Drying, in the sense in which the term could be applied to a flowering plant, does not take place, since the air-dry cells still contain in their protoplasts a considerable amount of moisture. This retention of moisture is presumably in great part due to the high concentration of the sap, although in view of the absence or paucity of obvious vacuoles it is possible that a large part of the sap is held adsorbed by colloidal constituents within the protoplast. The granules which are so characteristically produced as a reserve by all these terrestrial algae may be an expression of the peculiar conditions in the protoplasts as a result of the absence of vacuoles. Possibly, too, they form part of the mechanism by means of which moisture is retained in the cells.

EAST LONDON COLLEGE,
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Anatomical Structure of the Roots of Barley.

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With twelve Figures in the Text.

A BARLEY plant, if carefully removed from the soil when about six to eight weeks old, generally shows two distinct types of roots. The bulk of the underground system consists of long roots which branch throughout almost their entire length, but there are in addition a certain number of 'unbranched' roots. Most of these spring from one of the nodes above the grain and are therefore of later origin than the branched roots, which spring from the grain itself. They are of a very waxen white appearance and attain a length of several inches without giving off any laterals, but ultimately they branch and seem to approximate to the general root system.

The occurrence and behaviour of these white 'unbranched' roots were studied at Rothamsted in the summer of 1920, and material obtained then was preserved with a view to ascertaining whether there existed any difference between the anatomical structure of the branched and the unbranched type of root. Material was also obtained from a series of experiments in which barley was grown in water cultures. Very distinct white 'unbranched' roots are found in water cultures when the plants are removed from their solutions at the conclusion of the experiment, somewhat before maturity is reached. In addition to their white colour, 'unbranched' roots can be distinguished from branched roots by their greater thickness and by a much larger number of root-hairs. These latter are particularly noticeable in water cultures, where there is considerably less abrasion than occurs in the soil, but even in soil, if the plants are washed out as described in a previous paper,¹ it is always difficult to free the white 'unbranched' roots from the tiny soil particles which adhere very closely along the whole length of the root. In a branched root, this closely adhering soil is only found in the region immediately behind the root-tip, presumably where the root-

¹ Brenchley, W. E., and Jackson, V. G.: Root Development in Barley and Wheat under Different Conditions of Growth. *Ann. Bot.*, vol. xxxv, pp. 533-556, 1921.

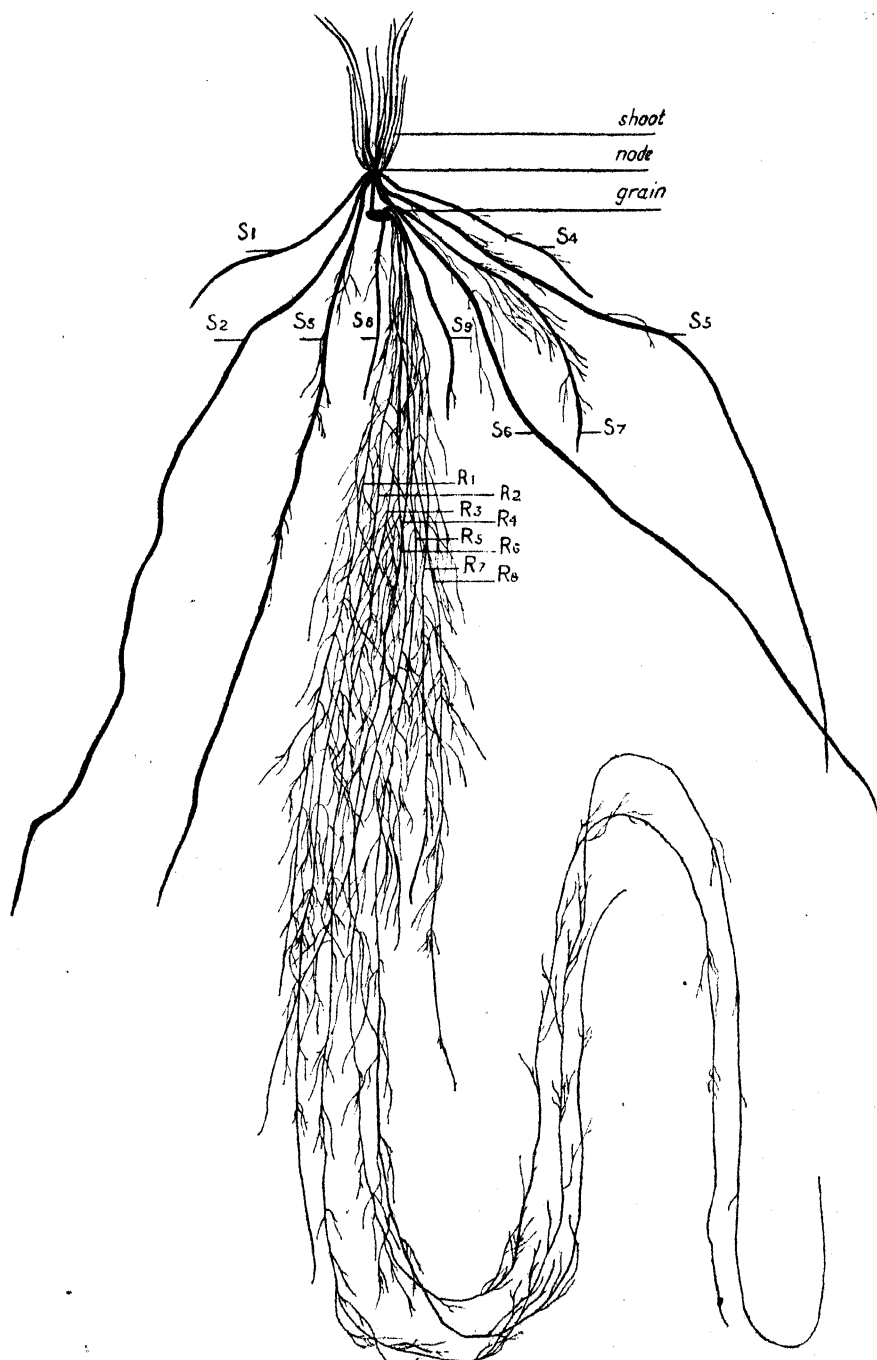


FIG. 1. Barley Plant. Drawn from actual specimen, $\frac{1}{2}$ natural size. R₁-R₈=branched roots; S₁-S₉= 'unbranched' roots.

[After Jackson, 1922]

hairs have not been rubbed away. This fact has been corroborated by examination of the washed roots under the binocular microscope, whereby the existence of abundant root-hairs from the tip of the 'unbranched' roots right up to their point of origin is made perfectly clear, while in the branched roots hairs are found only for a short distance behind the root-tips of the main roots and their lateral branches.

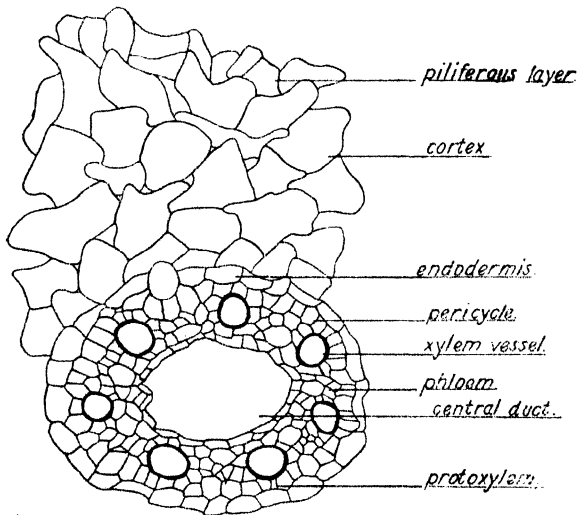
The general appearance of the root system of a plant bearing typical branched and 'unbranched' roots is shown in Fig. 1. In this case there are two short 'unbranched' roots, S_8 and S_9 , coming off from the grain, though they do not arise from quite the same point as the typical branched roots, R_1 – R_8 , of the primary root system. The majority of the 'unbranched' roots, S_1 – S_7 , spring from a node half an inch above the grain. These roots exhibit various stages of development, from the totally unbranched type S_1 to the type S_7 which is still markedly thicker than any of the roots of the primary system, but also bear a good number of laterals. Careful study of the literature on roots has failed to reveal any work on the anatomy or on the functions of these 'unbranched' roots, although the phenomena of 'white roots' is thoroughly well known to the farmer. There is undoubtedly a connexion between the tillering of a plant and its formation of 'unbranched' roots. From a series of pot cultures in which barley was grown, two plants were washed out each week and their root systems examined. Tillering was observed in all the plants just after the first 'unbranched' roots had appeared. This is also found to be the case in winter-sown wheat and oats growing in the field. During the winter the plants are growing very little, but in the spring they begin to push forward, and if a plant just starting to tiller is examined it is found that white 'unbranched' roots are practically always present. These roots appear also in the field barley, but this being a spring-sown crop the 'unbranched' roots appear at a much earlier stage in the plant's history than in either wheat or oats. That farmers recognize the existence and importance of these 'unbranched' roots is clear from the general practice of horse-hoeing a wheat crop until the 'change of root', which is really the formation of the white roots.¹ After this has happened it is considered dangerous to horse-hoe, as it would probably involve injury to the newly-formed roots, most of which it must be remembered are nearer the surface of the soil than are the primary roots, since they spring from the node above the grain.

The present paper embodies the results obtained from an anatomical investigation of the two types of roots as found in barley plants grown in soil and in water cultures.

¹ The term 'change of root' seems to be of general occurrence among farmers, but no reference to it has been traced in any of the books on agricultural practice.

ANATOMY OF BRANCHED ROOTS.

Young roots. A transverse section of a young barley root examined seven days after sowing, when the root was about 1 in. in length, presents the appearance shown in Fig. 2. The middle of the root is occupied by a kind of duct bordered by thin-walled cells. The endodermis bounding the stele is distinguishable as a row of tangentially elongated cells, with their walls as yet unthickened. Immediately within the endodermis lies the pericycle, consisting of radially elongated cells, the continuity of which is



Barley.

T.S. Young branched root. (x105) [After Jackson, 1922]

FIG. 2.

broken opposite each of the seven xylem groups, where the protoxylem elements abut directly on the endodermis. In addition to the smaller cells of the protoxylem each xylem group contains one large vessel. Alternating with the xylem are the phloem groups, the cells of which are at this stage not easily distinguishable from the rest of the thin-walled ground tissue. Outside the endodermis are four to six layers of large thin-walled cortical cells, bounded by the piliferous layer. The endodermis and all the tissues within it consist of cells packed tightly together, so that no intercellular spaces occur. The same is true for the piliferous layer, but in the cortex small spaces do occur between the cells. These spaces are not entirely due to shrinkage as they are found in fresh material.

Intermediate stage between a young and an old root. A root slightly older than that shown in Fig. 2 shows essentially the same structure, the chief difference being in the pericycle and endodermis, where the cells show signs of thickening. In the pericycle this thickening affects the whole cell wall, but in the endodermis it affects chiefly the inner and radial walls, and it is most noticeable in the cells lying in the zone between the xylem groups. This seems to suggest the formation, at least in the comparatively young roots, of passage cells, as described for *Allium* and various other plants by

Haberlandt,¹ where the endodermal cells opposite the protoxylem remain unthickened and serve as the connecting link between the central cylinder and the cortical parenchyma.

Fully developed roots. Fig. 3 shows the appearance of a transverse section of an old root. The stele here consists of a strongly marked cylinder of much-thickened tissue, and the central duct is bounded by a thick wall and closely resembles a xylem vessel. Such an axile vessel occupying the centre of the root is described by Haberlandt² for the primary roots of Graminaceae and certain other Monocotyledons, and by Kroemer³ for *Zea Mais*, but is not mentioned by Jeffrey,⁴ who states that for the 'mass of monocotyledonous roots' the 'central region of the root is occupied by a well-marked pith'. The number of xylem groups seem to be typically from six to eight; in the root shown in Fig. 3 there are seven groups each containing one large vessel, and alternating with seven groups of phloem, all the intervening tissues consisting of thickened cells.

The endodermis is exceptionally thick-walled, a condition which appears constantly throughout the old branched roots. This much-thickened endodermis is a characteristic of several monocotyledonous plants.

It is illustrated for *Allium* by Haberlandt,¹ for *Zea Mais* by Kroemer,³ for *Oryza* by Nägeli and Leitgeb,⁵ and for *Dracacna* by Lindinger.⁶ Sections taken at different levels through a branched root 28 in. in length reveal the stages shown in Fig. 2 and 3. Immediately behind the root-tip the structure is like that of the young root in Fig. 2, while in the middle portion of the root and near the grain the thickened stele of Fig. 3 is found.

A longitudinal section of an old root shows that the central duct is

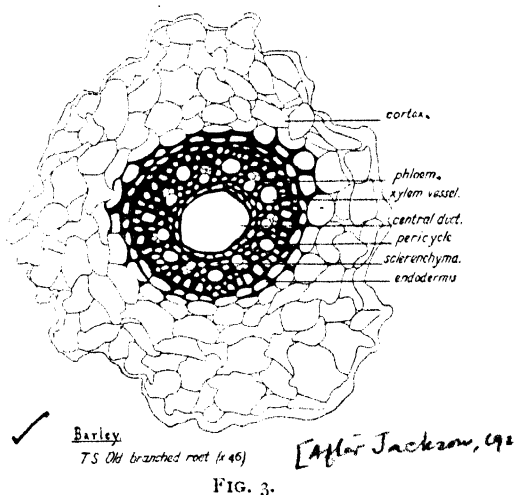


FIG. 3.

¹ Haberlandt, G.: Physiological Plant Anatomy, p. 370. Trans. from 4th German ed. by Montagu Drummond, 1914. Macmillan & Co., Ltd., London.

² Loc. cit.

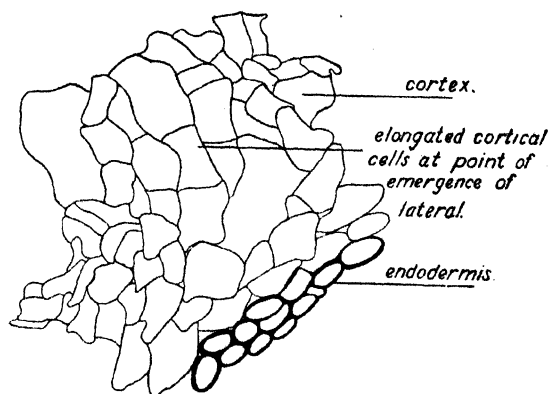
³ Kroemer, Karl: Wurzelhaut, Hypodermis und Endodermis der Angiospermenwurzel. Bibliotheca Botanica, Stuttgart, 1903, Heft 59, pp. 151, 6 Taf.

⁴ Jeffrey, Edward Charles: The Anatomy of Woody Plants, p. 158. Univ. of Chicago, 1917.

⁵ Nägeli, C., and Leitgeb, H.: Entstehung und Wachstum der Wurzeln. Beiträge zur wissenschaftlichen Botanik, Heft iv, pp. 73-160, Taf. xi-xxi.

⁶ Lindinger, L.: Monokotylenwurzel. Bot. Centralbl., Jena, Beiheft 19, Abt. 1, 1905, pp. 321-58.

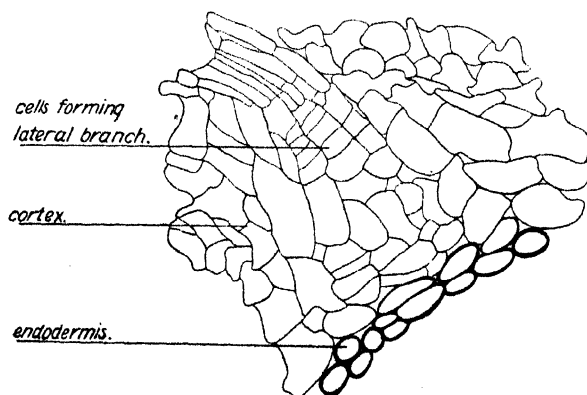
of the nature of a vessel with scalariform thickening. The transverse septa of the cells forming the vessel are still present and appear as very thin parenchymatous walls. On either side of the central duct lie several rows



Barley.

T.S. Root through point of emergence of lateral. (a) (branched type) (x110)

FIG. 4.



Barley.

T.S. Root through point of emergence of lateral (b) (branched type) (x110)

FIG. 5.

of fibrous cells with pointed ends. Among these are the vessels of the xylem groups, which are generally spirally thickened. Outside this group of cells lie the pericycle and endodermis, which appear as comparatively

thin-walled cells with square ends, and these are separated from the piliferous layer by the parenchymatous cells of the cortex. The xylem and phloem lie along different radii, therefore a longitudinal section passing through the xylem does not pass through the phloem, so that phloem tissue does not appear in this section.

These primary roots emerge from the grain, and soon reach a length of about 5 in., when they begin to branch freely. The origin of these lateral branches is in some sections shown to be a group of meristematic cells abutting on the endodermis. These cells are full of cell contents and take up any stain very readily. In this condition they resemble the formation of lateral roots in *Oryza*, as described by Nägeli and Leitgeb.¹ The meristematic stage is, however, soon over; the lateral root grows out, and most frequently adjacent sections through the point of emergence present the appearance shown in Figs. 4 and 5.

Branched roots are found coming from the node as well as from the grain. Those from the grain are present in the embryo itself and form the primary root system, but those from the node are adventitious in origin. The structure, however, is the same, both for the primary and for the adventitious roots, and in each case transverse sections show the typical thick-walled endodermis and single large axile duct surrounded by much-thickened tissue.

ANATOMY OF 'UNBRANCHED' ROOTS.

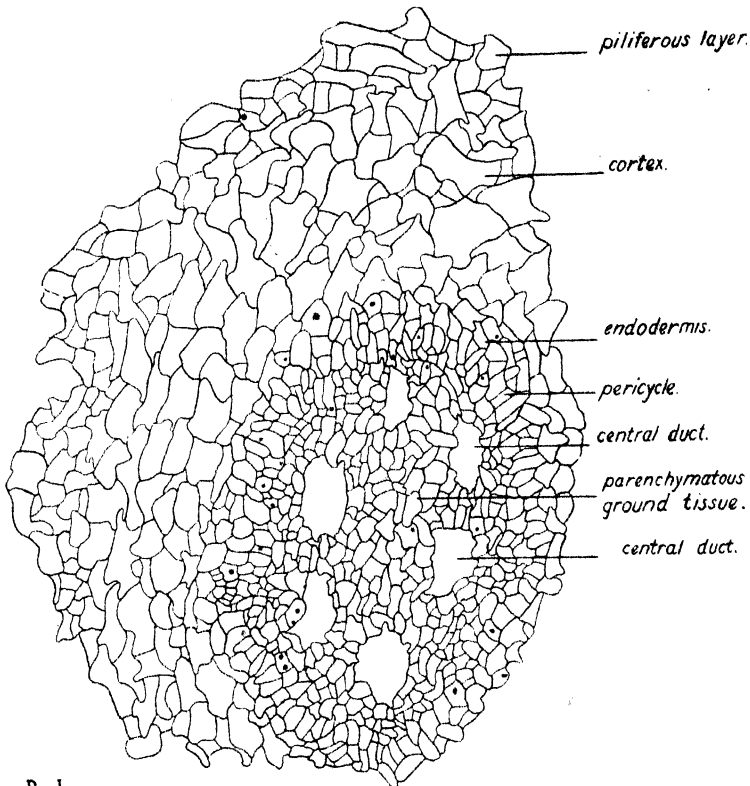
Young root. The structure of the 'unbranched' roots differs considerably from that of the branched type. The appearance of a section of a typical young 'unbranched' root is illustrated in Fig. 6. At this stage the whole of the stelar tissue is thin-walled; the central region consists of a core of parenchymatous cells traversed by several large ducts arranged in a circle. In this case six ducts are present, but the number varies for different roots. The xylem and phloem are not yet differentiated, so that outside the circle of ducts lie three or four rows of parenchymatous cells similar to those of the central core. These are bounded by the pericycle, which is distinguishable by its radially elongated cells. The cells of the endodermis are elongated tangentially, and are thus distinguished from the pericycle on the inside and from the cortex on the outside. Eight or nine rows of parenchymatous cells form the cortex, and these are bounded by the thin-walled cells of the piliferous layer.

Intermediate stage between a young and an old root. The structure of a slightly older root is shown in Fig. 7, which illustrates a section taken at a point about half an inch behind the tip of an 'unbranched' root three

¹ Nägeli and Leitgeb: loc. cit.

28 *Jackson.—Anatomical Structure of the Roots of Barley.*

inches in length. The central region of the stele consists of thin-walled parenchyma, and in this root four large ducts are present, two of which are almost completely divided into two separate cavities, so that in the older part of the root six fully developed ducts are found. The xylem groups, each containing one large thickened vessel, are arranged in a circle outside that formed by the ducts. They are separated by thin-walled parenchyma,



✓ Barley.

part of a T.S. of a
Young "unbranched" root. (x105)

[After Jackson, 1922]

FIG. 6.

the phloem at this stage not being differentiated from the rest of the ground tissue. The pericycle consists of typical radially elongated, and the endodermis of tangentially elongated cells, but in neither layer are any of the cell walls thickened. The cortex is composed of about seven rows of large parenchymatous cells, of which only the inner five rows are shown in the figure. The piliferous layer bounding the cortex also consists of parenchymatous cells rather elongated in the radial direction.

Fully developed roots. A still older root presents the appearance

shown in Fig. 8. In this case the central part of the stele is traversed by four large ducts separated from each other by thickened cells, but the actual boundary wall of each duct remains thin. Each xylem group contains one large vessel situated on the inner side of the protoxylem elements, and the groups are separated by small parenchymatous cells, among which the phloem cells are not easily distinguishable. The pericycle is composed of cells elongated radially and thickened uniformly on all the walls. Its continuity is interrupted opposite each of the xylem groups where the protoxylem elements abut directly on the endodermis. Here again passage cells occur, for the endodermis cells adjoining the

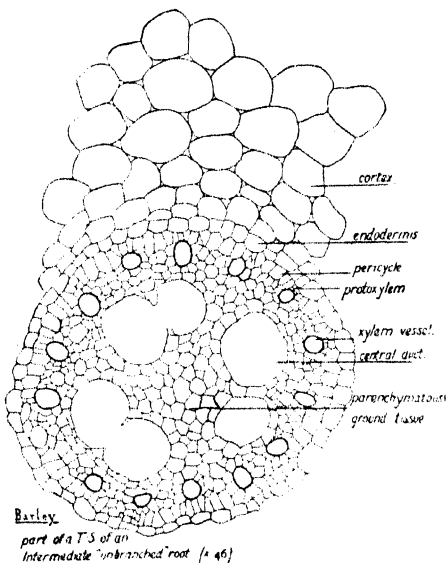


FIG. 7.

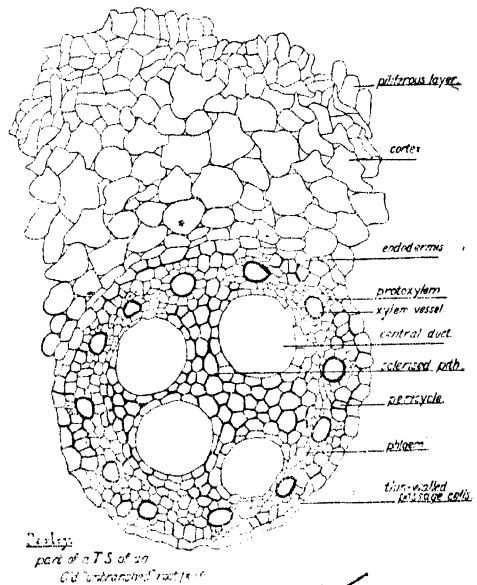


FIG. 8.
(After Jackson, 1922)

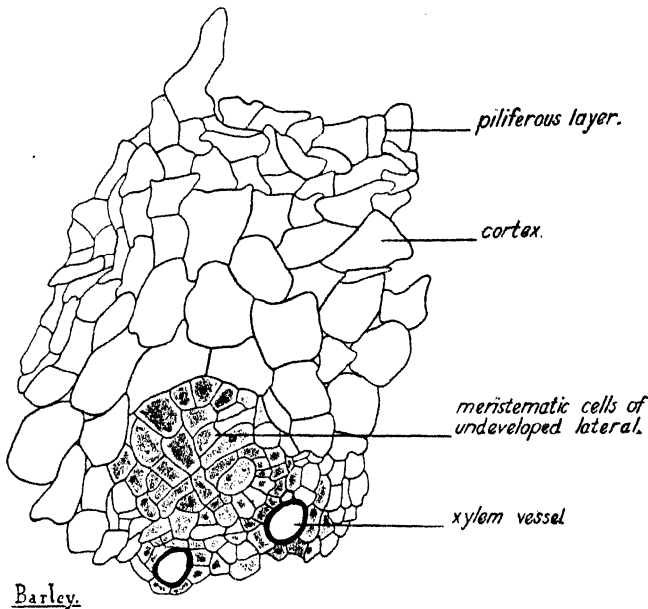
protoxylem elements are entirely unthickened, whereas thickening does occur on the radial walls of some of the other endodermis cells. Outside the endodermis are six to eight rows of large parenchymatous cortical cells, bounded by a well-marked piliferous layer.

A longitudinal section of an 'unbranched' root shows that the ducts consist of wide elongated parenchymatous cells, the transverse septa being of the same thickness as the longitudinal walls. When two ducts appear in the section they are separated by several rows of cells, some of which have square, while others have pointed end-walls. The former are the thin-walled cells on the outer edge of the central stelar tissue, while the latter are the sclerized cells of the actual centre. The outer edge of the duct is bounded by a band of parenchymatous cells with square end-

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walls, and among these occur typical spiral and scalariform vessels. This layer of cells is bounded by the thin-walled elements of the pericycle and endodermis, and outside these are the parenchymatous cells of the cortex and of the piliferous layer.

Incipient laterals are present in a typically 'unbranched' root. This is shown in Fig. 9, where the continuity of both the pericycle and the endodermis is entirely broken by a group of meristematic cells in process of forming a lateral branch. This condition is of frequent occurrence and shows up very clearly, as the meristematic cells readily take up the stain.



Barley.

*part of a T.S. of an "Unbranched" Root,
showing an undeveloped lateral. (x110)*

FIG. 9.

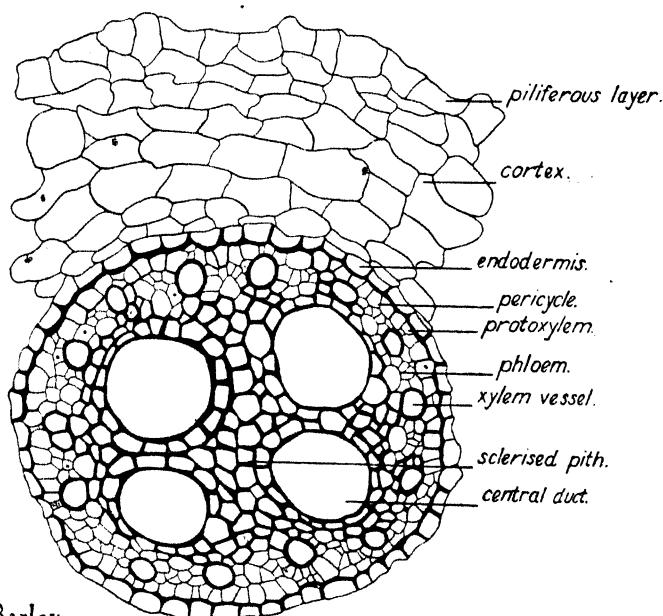
It closely resembles the appearance depicted by Nägeli and Leitgeb¹ for the development of lateral roots in *Oryza*. These incipient roots remain dormant in their meristematic condition for some time, and only push out when the root is changing from the 'unbranched' to the branched type.

The 'unbranched' roots from the grain are also adventitious, and on sectioning them it is found that their structure is strictly comparable with that of the 'unbranched' roots from the node, a typical section showing a stele with characteristic parenchymatous central region and three ducts.

Development of roots from 'unbranched' into branched type. The ultimate fate of these 'unbranched' roots is that they give off laterals and

¹ Nägeli, C., and Leitgeb, H. : loc. cit.

approximate more and more to the branched type. At the same time the internal anatomy undergoes certain changes. Fig. 10 represents a section of a root of this transition type. The most noticeable feature is the increased development of thickened cells in the central tissue of the stele. The endodermis is also thickened, though not to such a great extent as in the typical branched root. The only cells which are still thin-walled are those of the pericycle and those between the several xylem groups. The phloem cells also become more differentiated and assume the appearance of the corresponding tissue in the branched roots.



✓ Barley.

*part of a T.S. of a
Transition root.
(taken near grain). (x218)*

[After Jackson, 1922]

FIG. 10.

The question then arises as to what happens to the newly formed parts of these transitional roots. Fig. 11 is drawn from a section taken near the tip of a root, which was obviously of the 'unbranched' type but carried a number of laterals. A section near the grain of this root shows essentially the same structure as that of Fig. 10, that is, there are five large central ducts and a considerable number of thickened cells in the stele, but here at the tip there are only two very large central ducts and the development of thickened tissue is still more marked. The phloem is also well differentiated and the pericycle and endodermis are thickened, especially in the zones between the xylem groups. In the water-culture material several roots

were found in which a section near the grain shows five large ducts traversing a stele in which all the tissues are thickened, while near the root-tip only one central space occurs in a stele of the type found in the young branched roots.

The outstanding differences between the typical 'unbranched' roots and the roots which were originally 'unbranched' but have assumed a branched habit is that in the former the young root shows the characteristic unthickened stele with its central region traversed by four to six ducts, while the latter possesses a stele with a considerable quantity of sclerized

tissue and only one, or at the most two, central ducts. The older parts of the root resemble each other in general structure, but the stele of the 'unbranched' root shows less thickening than that of the transition root.

Comparison of the anatomy of the branched and the 'unbranched' roots. A certain number of measurements were made of the sections of the two types of roots obtained from five sets of differently manured pot cultures. The material available was limited, but, with the exception of the superphosphate and potash manuring, measurements were obtained of two branched and two 'unbranched' roots from all the manural types. Two diameters at right angles to each other were measured for the

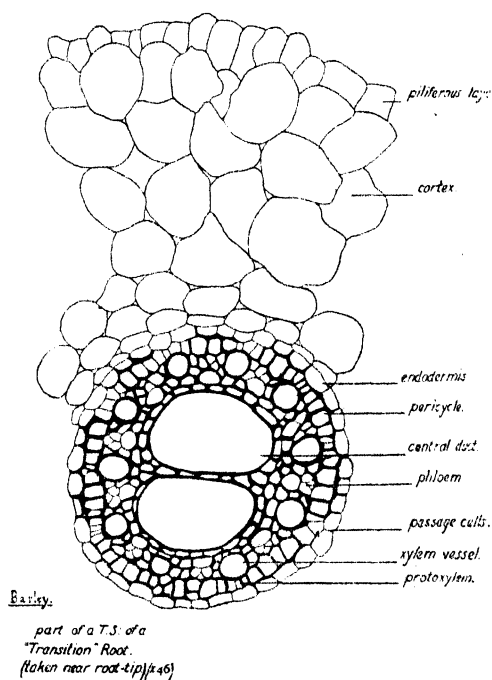


FIG. 11.

whole root, the stele, and for the central duct or ducts. It must be remembered that, when this material was being preserved, it was not realized that the 'unbranched' roots ultimately resembled the branched type, so that in certain cases roots which were originally of the 'unbranched' type were preserved as typical branched roots. The measurements of these transition roots together with those of their corresponding true 'unbranched' roots are collected together in Table II, while in Table I are the measurements of the true branched and 'unbranched' types. It will be noticed that in both cases where originally 'unbranched' roots were taken as branched roots, the plants had been treated with superphosphate, and this is a manure which tends to hasten the formation of 'unbranched' roots, so that at

TABLE I.

Manurial Treatment.	Root Type. Br. = branched. Unb. = unbranched.	Diameters taken at Right Angles.		Number of Ducts.	Number of Xylem Groups.	Area Ratios.	
		Whole Root.	Stele.			Whole Root. Stele.	Whole Root. Ducts.
Unmanured	Br.	mm. 0.39	mm. 0.16	1	7	4.9	40.8
"	"	0.34	0.16	1	7	3.4	24.4
"	Unb.	0.55	0.29	5	0	3.7	9.8
"	"	0.57	0.29	6	0	4.2	111.4
Nitrate	Br.	0.39	0.15	1	7	5.2	44.9
"	"	0.40	0.16	1	7	5.8	52.3
"	Unb.	0.69	0.27	4	15	5.7	33.7
"	"	0.63	0.29	5	15	4.8	22.4
Superphosphate and nitrate	Br.	0.35	0.14	1	8	4.0	28.6
"	"	0.43	0.28	1	6	4.9	35.5
"	Unb.	0.81	0.60	4	14	6.3	43.6
"	"	0.61	0.58	5	16	4.6	33.8

In the table the actual figures for the roots of each plant are given, while in the paper the averages from the two plants in each set are compared.

TABLE II.

Manurial Treatment.	Root Type. Br. = branched. Unb. = unbranched.	Diameters taken at right angles.		Number of Ducts.	Number of Xylem Groups.	Area Ratios.	
		Whole Root.	Stele.			Whole Root. Stele.	Whole Root. Ducts.
Superphosphate	Br.	mm. 0.61	mm. 0.26	4	14	4.6	23.8
"	"	0.52	0.25	4	14	4.2	19.3
"	Unb.	0.79	0.35	5	14	5.0	19.2
"	"	0.68	0.28	4	12	5.1	25.1
Superphosphate and potash	Br.	0.56	0.28	4	12	3.7	2.5
"	"	0.45	0.20	1	7	3.9	44.9
"	Unb.	0.75	0.38	5	15	4.2	30.2
"	"	—	—	—	—	—	—

In the table the actual figures for the roots of each plant are given, while in the paper the averages from the two plants in each set are compared.

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a given time a plant with superphosphate would have more and older 'unbranched' roots than an unmanured plant of the same age. Hence it is obvious that in such a plant there would be more chance of preserving as branched roots those which were originally of the 'unbranched' type.

Considering only the figures in Table I, there is quite a marked difference between the sizes of the roots of the two types, the range for the diameters of the branched roots being 0.26 mm.—0.40 mm. against 0.38 mm.—0.71 mm. for the 'unbranched' roots. The stelar diameters also show a corresponding difference in size, the range being 0.15 mm.—0.16 mm. for the branched root against 0.19 mm.—0.29 mm. for the 'unbranched' roots. It may be observed that for the branched and 'unbranched' types respectively, the size of the stele is remarkably uniform in the roots taken from all the different types of manuring.

An attempt was made to get some estimate of the ratio of (a) the area of the stele (in cross-section) to the area of the whole root; (b) the area of the central ducts to the area of the whole root; and (c) the area of the central ducts to the area of the stele. Since the different areas to be measured were neither true circles nor true ellipses, it was impossible to secure actual figures. The ratios, however, can be obtained fairly accurately by taking as area the product of the two diameters, since the area of an ellipse is proportional to that of its escribed rectangle. In cases where there are several central ducts, the sum of the products of their several diameters is taken as representing the total central space. The ratios of stele to whole and of central space to whole did not seem to follow any definite rule. This may possibly be explained by the fact that all the cortical tissues had shrunk considerably owing to their lengthy immersion in alcohol. Of course this shrinkage also affects the total diameter measurements, but as these figures can only be very preliminary, it was thought justifiable to include them as a slight indication of the differences existing between the two root types. The material was all treated in exactly the same way, so that presumably the shrinkage of the different sets of material would be approximately the same, and the figures are therefore comparable, though not true measures of the diameter of the fresh roots. This shrinkage, however, did not affect the stele—except perhaps in the case of the very thin-walled cells of the 'unbranched' roots in the unmanured series. There is a fairly well marked difference between the ratios of stele to central space in the branched and in the 'unbranched' roots, the ratio being persistently higher in the former than in the latter, as would be expected from the large amount of central space in the 'unbranched' roots.

Summary of the chief points of difference in the morphology and anatomy of the branched and 'unbranched' roots. 1. The 'unbranched' roots are thick and very white. They grow to a length of several inches without any

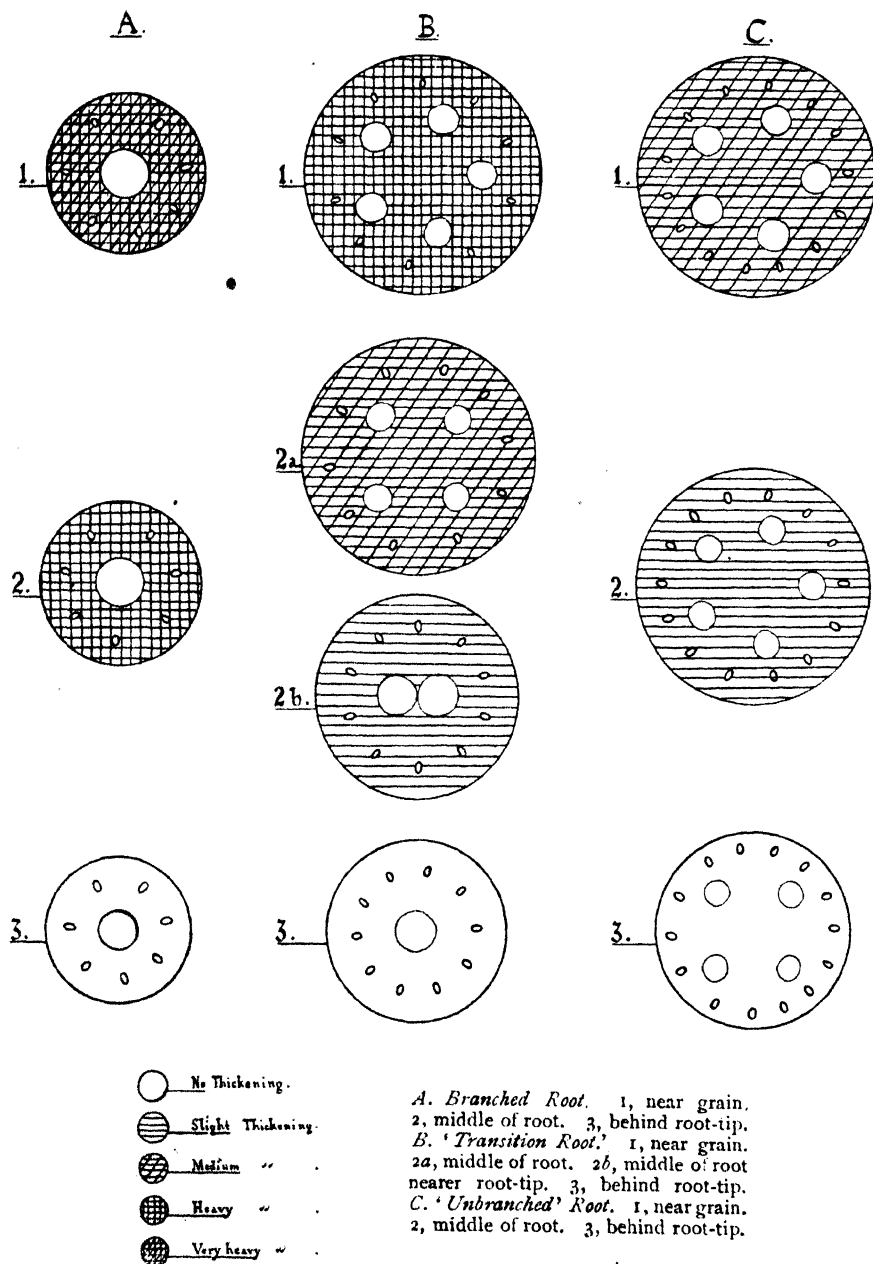


FIG. 12. Barley. Diagrams showing stelar structure of the different types of roots.

laterals; the branched roots are thin and give off laterals when only an inch or two long.

2. Abundant root-hairs exist all along the 'unbranched' roots, but only behind the growing points of the branched roots.

3. The central region of the stele of an 'unbranched' root is traversed by four to six ducts with comparatively thin walls, while in the branched root there is only one central axile duct bounded by thick-walled cells.

4. In an 'unbranched' root the endodermis, pericycle, and all the outer tissues of the stele, with the exception of the xylem vessels, are thin-walled; in a branched root all the stelar tissues, especially the endodermis, are very much thickened.

5. The number of xylem vessels varies from twelve to sixteen in the 'unbranched' root, but from six to eight in the branched root.

These differences are represented diagrammatically in Fig. 12.

Functions of the 'unbranched' roots. Branched roots having identically the same structure as that of the primary branched roots are sometimes found springing from one of the nodes above the grain. Such a root must be of adventitious origin, and it is therefore certain that the differences in structure existing between the 'unbranched' and the branched roots are not due merely to the fact that the former are adventitious while the majority of the latter are present in the embryo and spring from the grain. It seems probable that the 'unbranched' roots have some special function for which they are adapted by their structural peculiarities.

It might be suggested that they are of the nature of contractile roots, and serve to pull the plant down to a more favourable position in the soil. But contractile roots generally show a very distinct wrinkled surface caused by the cortical cells of the roots becoming, by their turgescence, more stretched transversely than longitudinally, and such an appearance is not found in the 'unbranched' roots. Further, these roots are always present in water cultures where contractile roots would certainly not be needed.

Another suggestion is that they are a special system developed to provide extra support for the plant when the parts above ground are increasing in weight. The 'unbranched' roots spread out considerably and form a network just beneath the soil surface, so that they are in a position to give good support to the plant. They are then physiologically comparable with the stilt- or buttress-roots occurring in *Pandanus*, *Rhizophora*, &c., and still more comparable with the adventitious roots which arise from the lower nodes of the stem of *Zea Mais*. Haberlandt,¹ following Warming's description of the stilt-roots of *Rhizophora Mangle*, states that they are of a stem-like structure. The middle of each root is occupied by a large pith surrounded by alternating groups of xylem and phloem, while thick-walled mechanical cells are found on the medullary side of the water-

¹ Haberlandt: loc. cit., p. 188.

conducting tissue. For the adventitious roots of *Zea Mais* Haberlandt describes a fibrovascular cylinder enclosing a wide core of pith surrounded by alternating groups of xylem and phloem. Very wide vessels disposed in a ring are conspicuous in the xylem groups, and the endodermis is thick-walled. Further, there is a zone of thick-walled parenchyma found in the cortex just within the piliferous layer. The 'unbranched' roots of barley certainly have a central pith, though traversed by ducts, but there is no development of thick-walled tissue to correspond to that found in both *Rhizophora* and *Zea*. On the other hand, farmers hold that if a horse-harrow is used among cereals after the 'tillering' or 'unbranched' roots are well established, the plants do not stand so well and are apt to lodge badly. If the 'unbranched' roots are to render support to the plant, then it would be expected that their mechanical tissues would be developed at least as much as, if not more than, those of the branched roots, since the latter certainly do support the plant. Some idea of the mechanical strength of a root is given by the ratio of the area of the stele to that of the central space. Referring to Table I, it is seen that in the unmanured and in the nitrate only plants this ratio is considerably higher for the branched than for the 'unbranched' roots, but in the superphosphate and nitrate plants the ratio is the same for both root types. Presumably then, in the last-named manurial series, the support offered by the 'unbranched' is equal to that offered by the branched root, and this may be significant in view of the fact that superphosphate manuring checks the lodging tendency which occurs in plants receiving only nitrogenous fertilizers.¹ It is therefore probable that the 'unbranched' roots do serve to some extent as 'buttress' or supporting roots.

Most probably the main use of the 'unbranched' roots is to be found in connexion with the plant's nutritive supply. The roots appear when the plant is beginning to grow vigorously, and consequently when it is needing a good supply of water and food which must be obtained chiefly from the soil. The 'unbranched' roots are so constructed that they are specially adapted to meet such a demand. They are invested with root-hairs throughout their entire length, so that their absorptive area is considerably larger than it is in the branched roots, where absorption can only take place in the parts behind the growing root-tips, where the root-hairs are still functioning. The number of large xylem vessels is increased, so that there are more passage-ways along which the water can travel with ease. The large ducts in the pith are also probably used for the translocation of water, while the unthickened condition of practically all the stelar tissues allows water to pass from cell to cell with considerable rapidity.

It may be suggested that the development of lateral branches in the

¹ Purvis, O. N. : The Effect of Potassium Salts on the Anatomy of *Dactylis glomerata*. Journ. Agric. Sci., vol. ix, Part IV, Oct. 1919, p. 339.

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'unbranched' roots is influenced by light, since these roots occasionally come up to the soil surface and are consequently nearer the light than are the branched roots. That this is not the case is clear from the fact that ultimately lateral branches are developed; moreover, Newton¹ has shown that for terrestrial plants grown in water, branching takes place just as freely in the light as in the dark. The dormancy of the lateral branches is explained by the fact that the 'unbranched' roots develop when the plant is making vigorous growth and needs a plentiful supply of water with its dissolved nitrogenous and mineral constituents; if the roots were to produce laterals immediately, then thickening of the stelar tissue would follow and the translocation of water would become slower. On the other hand, when the laterals are dormant, the tissues of the stele remain comparatively thin-walled and water can very readily pass through the roots to the stem and leaves, and in addition the root-hairs are able to continue functioning along the whole length of the root.

The theory that the 'unbranched' roots are chiefly connected with the food- and water-supply of the plant receives further support from the fact that these roots are only formed during the early stages of the plant's vigorous growth. Researches on the development of root and shoot² showed that the formation of 'unbranched' roots had entirely ceased by the time the plant had finished its vegetative growth and was entering on its reproductive phase. At this period of the plant's history the nitrogen and ash constituents are migrating steadily from the straw into the grain,³ so that there is no need for a large root-absorbing area. On the other hand, if the 'unbranched' roots functioned chiefly as buttress-roots, the plant would need them even more when the heavy grain is being formed, but that is just the time when their development ceases. Therefore the most probable function for the 'unbranched' roots is to ensure a good supply of water, &c., when the plant is in a condition of strong vegetative growth.

SUMMARY.

1. The root system of a well-developed barley plant consists of two types of roots: (a) a thin branched type, and (b) a thick 'unbranched' type, with very abundant root-hairs.

2. A branched root possesses a much-thickened stele with a single large axile vessel and six to eight xylem groups all bounded by a very thick-walled endodermis. In an 'unbranched' root neither the endodermis nor the stelar tissues are thickened, the xylem groups number from twelve to sixteen,

¹ Newton, L. M.: Conditions which affect the Branching of Roots. Rept. Mich. Acad. Sci., Lansing, 1911, xiii, p. 200.

² Brenchley and Jackson: loc. cit.

³ Brenchley, W. E.: The Development of the Grain of Barley. Ann. Bot., vol. xxvi, No. ciii, July, 1912, pp. 913-19.

and the middle of the root consists of thin-walled pith cells traversed by four to six ducts.

3. The chief function of the 'unbranched' roots is probably to provide the plant with a plentiful supply of water and its dissolved food, at the time when vigorous growth is setting in. This function is provided for by :

- (a) abundant root-hairs ;
- (b) an increased number of large vessels and central ducts ;
- (c) the existence of a stele composed almost entirely of thin-walled elements.

In conclusion I wish to express my thanks to Dr. W. E. Brenchley for her very helpful and ever-ready advice throughout the whole of this work.



Some Observations on *Isoetes Drummondii*, A.Br.

BY

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With fifteen Figures in the Text.

*I*SOETES DRUMMONDII is a small terrestrial species that has been recorded in West Australia and Victoria. It has recently been found in several localities in South Australia, always growing in soil that is wet in the rainy season, though not submerged, and baked dry in summer.

The presence near Adelaide of an abundance of material of an *Isoetes* having a subterranean stock has afforded an opportunity to make observations in the field on certain points in the biological morphology of the plant and to study its method of spore dispersal.

The material has all been collected in South Australia, chiefly at Belair, near Adelaide, where the majority of the field observations were made. Much of the laboratory work was carried out in the Botanical Department, University of Adelaide, but it has been completed in the Cryptogamic Research Laboratory, University of Manchester, whilst holding an Honorary Research Fellowship of the University. My thanks are due to Prof. W. H. Lang, F.R.S., for facilities afforded me in his laboratory, for the generous way in which he has placed material of other species at my disposal for comparison, for his kind interest, and for helpful criticism.

FIELD OBSERVATIONS.

The conditions under which *Isoetes Drummondii* grows have been described recently;¹ it is sufficient to recall here that in South Australia it occurs in a region of winter rainfall; 80 per cent. of the total annual precipitation (which is about 29 in. at Belair) falls in the seven months April to October inclusive. During this period the weather is cool, and the light intensity is frequently diminished by cloud or mist (though there are many intervals of bright sunlight) and the soil is often saturated. In October the temperatures become higher, and the rainfall usually diminishes

¹ Osborn, T. G. B. : Trans. Roy. Soc., S. Aus., vol. xlii, pp. 1-12, 1918.

until in January and February it may be little more than 0.5 in. per month. The dry season extends from November to March, during which time the vegetative activity of all small herbaceous plants is at a standstill, the

temperatures high, the insolation often intense, and the soil baked hard. It is not usually until the close of May that, after several periods of heavy rainfall, the soil becomes thoroughly saturated and perennial herbs make their appearance.

The vegetative period for *Isoetes Drummondii* extends from the end of May or the beginning of June to November. During this time the plant forms a small rosette of from eight to twenty linear terete leaves, bright green and rather diaphanous, through which the septa of the four air-canals can be seen. Below ground the leaves collectively form a slightly bulbous structure (Fig. 1) composed of their closely imbricate, wide, membranous bases. This bulb-like base is colourless, and arises from a small trilobed stock which is buried about 2 cm. below the surface of the soil. The stock bears a number of colourless bifurcating roots arising from the three grooves. Numerous brown withered roots are found

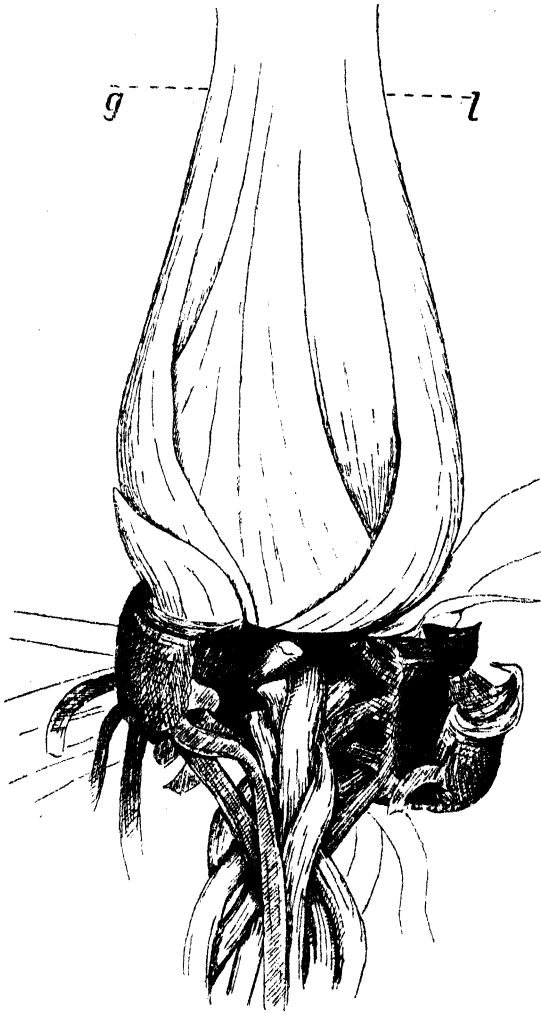


FIG. 1. Subterranean portion of a plant of *Isoetes Drummondii* collected towards the close of the growing season. Two lobes and the groove between them are visible. Each lobe bears withered roots of the previous growing season; the functional roots issue from the groove. The lobe to the right bears the older portion as a 'cap' partially detached.

on the lower surface of the lobes, but it is noticeable that the upper leaf-bearing surface has no remains of old sporophylls upon it (Fig. 2). With the advent of the dry season the leaves become yellow and rapidly wither;

their dry laminae may persist for some weeks, but ultimately become detached from the base somewhat below the soil level. Hence, in summer, plants can hardly be found even by careful search of small areas in which they are known to occur.

There is thus left below ground, during the dry season, the stock, bearing on its upper surface the somewhat cone-shaped mass of sporophyll

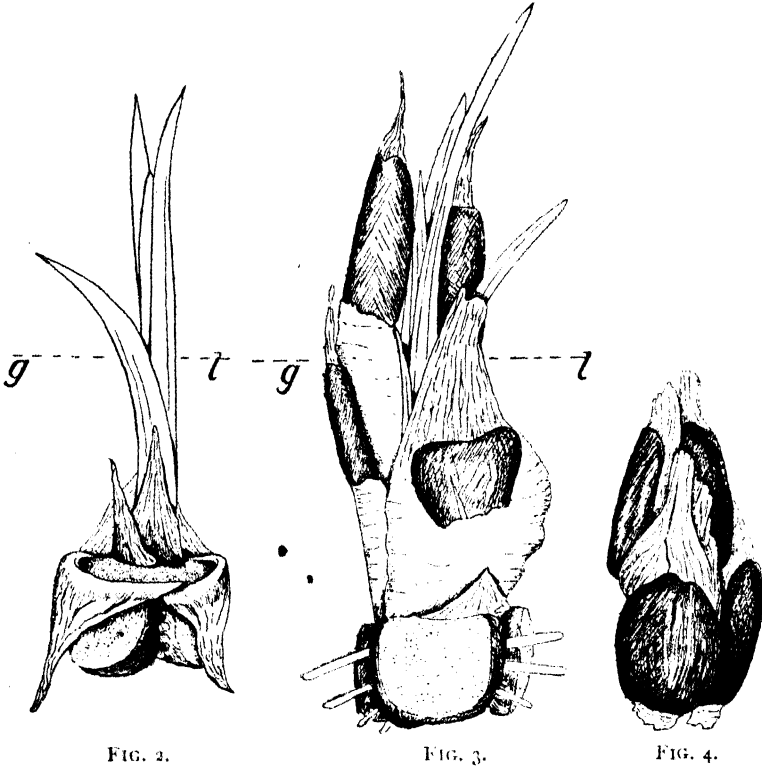


FIG. 2.

FIG. 3.

FIG. 4.

FIG. 2. Plant collected at the opening of the vegetative season. The upper leaf-bearing surface of the stock shows a clear ring between the scales that surround the young leaves and the outer withered bases of the previous season's sterile leaves, two of which are shown reflexed. Two of the lobes are visible, the 'scar surface' stippled. The roots are cut off close to the groove. $\times 3$.

FIG. 3. Plant collected in June at the stage of elevating the old sporophylls. The mucilage tissue at the bases is much swollen by water (cf. Fig. 2, which was drawn at the same time from a similar plant from which the mucilage and sporophylls had been removed). $\times 3$.

FIG. 4. Dry projectile-like mass of imbricate sporophyll bases, collected on surface of soil in June. Below it two dry contracted masses of mucilage can be seen: $\times 3$.

bases and the withered roots which dry up and die off completely at the beginning of the dry season. This condition persists for from four to five months. The stock has a hard brown coat formed of the sclerized outer cells over the whole of its surface, and the depressed apex is roofed over by three or four triangular imbricating scale-leaves, the cells of which are sclerized.

About the end of May or early June the first leaves of the new vegetative season appear, while previously, below ground, a new set of roots has begun to develop upon the stock. Coincidentally, or slightly before the appearance of the leaves, the bases of the previous season's sporophylls appear at the surface of the soil. These are forced upwards during such time as the soil is thoroughly sodden by the expansion of a mucilage tissue formed at their bases. In the field it is noticeable that the new leaves frequently appear from neat circular holes in the soil, rather like large worm-holes, which may contain a clear jelly. These holes are drilled by the sporophyll bases in their journey to the surface. In other cases the old sporophyll bases and the laminae of the new leaves appear above the surface of the soil at the same time (Fig. 3).

If rain continues after the projectile-like mass of sporophyll bases reaches the surface, it may fall apart, separating the individual sporophylls; but if a period of fine dry weather follows after the mass is exposed, it will dry up, forming a hard cone-shaped body (Fig. 4), and in this state may be blown or rolled away from the plant producing it. Ultimately the mass falls apart and the sporangia open by a tearing away of the walls from their attachment to the sporophyll. This only occurs during rain or immediately after, when the soil is thoroughly sodden, often showing a shimmer of surface water.

OBSERVATIONS ON THE STOCK.

Apart from its method of spore dispersal the study of *Isoetes Drummondii* has proved interesting in that it shows the behaviour of the stock of an *Isoetes* when grown under conditions with a sharp alternation of vegetative and resting seasons, conditions that are the reverse of the 'even growth' implied in the generic name.

If a plant be dug up and examined during the growing season the appearance of the stock is as seen in Fig. 1. The current season's leaves occupy the centre of the upper leaf-bearing surface of the stock. Externally to this are the projecting portions of the three lobes, which are composed of a series of nested scale-like caps, that usually break away upon the removal of the plant or on washing. The caps show no remains of the sporophylls that were produced on their leaf-bearing surface; all that remains of the leaves is two or three scales or the rotting bases of the few sterile leaves formed first in each season's growth.

The sides and lower surface of the stock bear numerous roots (Fig. 5). The majority are dead and brown; only those that arise from the centre of each groove are white and functional.

The structure of the lobe is of interest. It shows clearly the three types of surface distinguished by Lang,¹ viz. the leaf-bearing surface the

¹ Lang, W. H.: Mem. and Proc. Manchester Lit. and Phil. Soc., lix, No. 3, p. 9, 1915.

split surface of the groove, and the scar surface. This last in aquatic species is more or less irregular, formed by decay or removal of the distal ends of the lobes after the leaves and roots borne on that region of the stock have ceased to function.

In *Isoetes Drummondii* the lobe is formed of a series of nested caps, representing successive season's growth. Each cap is bounded externally by thick-walled brown cells, bears the remains of roots, foliage-leaves, and scales of a previous season upon the lower and upper surfaces, and has also two scar surfaces. To the outer of these the more distal cap was attached,

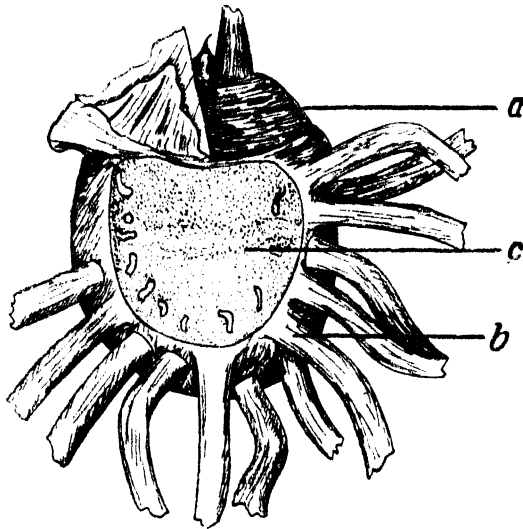


FIG. 5. A single 'cap' or desquamated stock lobe. *a*, the leaf-bearing surface, shows externally (i.e. nearer the observer) the remains of sterile leaves, some being cut away on the right, and internally two scale-leaves that protected the growing apex. The surface between was occupied by sporophylls, three scars formed by the leaf-traces of which can be seen. *b*, the root-bearing surface, with remains of one season's crop of roots. *c*, the distal 'scar' surface, to which a similar 'cap' formed the preceding season was attached, showing withered remains of root-traces of previous season.

while the whole proximal scar surface of the cap is composed of dead parenchyma cells. In this manner the old cortex of both leaf- and root-bearing portions of the stock is sloughed annually, giving rise to the caps, but it is not removed, since the soil holds it in position. A. Braun¹ noted this annual desquamation of the lobes of the stock, though he had not the knowledge of the plant in the field to connect the phenomenon with the seasonal changes that occur there.

'Cap' Formation on the Lobes.

The formation of a cap can most easily be followed in a series of median vertical sections of the stock taken at different seasons. Fig. 6, *a*,

¹ Braun, Alex.: Monatsbericht d. K. Akad. d. Wiss. Berlin, 1868, p. 543.

represents a vertical section of a stock in the plane of one lobe and groove, collected during the vegetative season. It is seen that the functional starch-packed parenchyma of the stock is roughly semicircular in section. The starchy tissue is limited on the outside by a narrow band of cells (one or two wide), the walls of which are in process of becoming thickened; this band lies two or three cells below the external surface. The lobe lying on the left-hand side of the figure is no longer a part of the storage tissue of the stock. It is composed of empty parenchyma cells, which at the distal portion have

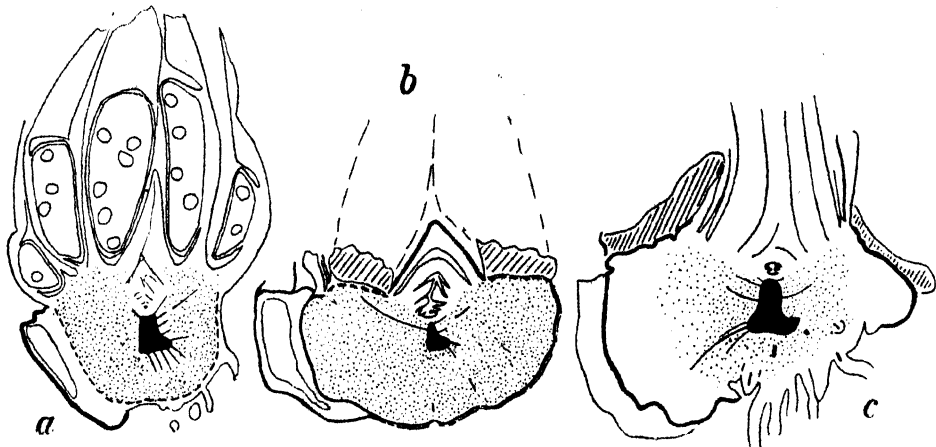


FIG. 6, *a*, *b*, and *c*. Series of vertical sections of stocks at different seasons cut in plane of one lobe and groove. Camera lucida outlines; with the magnifications employed, the layer of prismatic tissue around the main xylem mass could not be indicated. *a* shows stock at height of vegetative season. Leaf-bearing surface has megasporophylls and internally parts of two other leaves, then developing scales and leaf rudiments arching over depressed apex. Parenchyma of stock packed with starch; a short distance below surface a layer of cells is becoming thickened (broken line), eventually this completely cuts off roots. To left one 'cap' of lobe, its cells depleted of starch, torn apart and crushed. $\times 4.3$. *b* shows stock at beginning of dry season. The roots have died off, and starch-packed tissue of stock is bounded externally by sclerized cells. Sporophyll bases removed for sectioning, but outline indicated; mucilage tissue obliquely shaded; below this, position of thickened cells (not yet developed) shown by broken line. Apex and leaf rudiments protected by two scales, the outer only thickened. To left a 'cap' completely cut off from stock. $\times 6.5$. *c* shows stock at opening of vegetative season, when sporophylls were being shed. New season's leaves expanding, position of previous season's sporophylls shown by mucilage (oblique shading). Amount of starch is greatly diminished, particularly below old sporophylls. The formation of new leaf- and root-bearing surfaces has ruptured peripheral sclerized cells; the expansion of these surfaces will crush and distort the empty parenchyma, which will eventually be cut off as caps. Position of cap (not shown in preparation) indicated to left by light line. $\times 5.3$.

become torn apart, while at the proximal side they have become crushed and distorted. On its free surfaces the lobe is limited by hard sclerenchymatous tissue. The whole of the parenchyma composing the lobe is moribund and in process of isolation from the stock by the layer of thick-walled cells that is forming around the storage tissue.

In Fig. 6, *b*, a similar section of a stock is seen at the close of the vegetative season. The peripheral sclerenchyma is continuous except below the mucilage tissue of the leaf-bearing cortex, across which it extends ultimately as far as the scales protecting the apex. The apex is roofed over in this

specimen by two triangular imbricating scales, only the outer of which was sclerized when the specimen was collected. For sectioning it was necessary to remove the tough sporophyll bases and sporangia from the upper surface. Their attachment is shown by the mucilage tissue produced at their bases. The whole of the living parenchyma is packed with starch, and the stock at this stage is merely a perennating organ, its vegetative activity being suspended.

Fig. 6, *c*, shows the condition of such a stock about six months later, when the leaves and roots of a new vegetative season have appeared, and the sporangia of a previous one are being shed. The development of new leaf- and root-bearing surfaces has caused a rupture of the continuous sclerenchyma layer. This new growth has taken place at the expense of the starch, &c., stored in the outer region of the stock. This is now almost depleted of plastic substances at its periphery. The development of the cortex in the new leaf- and root-bearing regions forces the older parenchyma outwards, since the amount of elongation of the axis between leaf- and root-forming meristems is negligible. But, since the whole structure is subterranean and subjected to pressure by the soil on all sides, the lateral expansion at the centre causes distortion and crushing of the moribund distal portion, which, because the cells have ceased to grow and keep pace with the increasing circumference, becomes torn asunder and forms projecting lobes. These distal portions soon become cut off by sclerenchymatous tissue (cf. Fig. 6, *a*) and yet another cap is added to the lobe.

Thus it is seen that each annual set of caps represents the whole of the leaf- and root-bearing cortex of one growing season.¹ The annual desquamation of these caps follows because of the sharp alternation of vegetative and resting seasons imposed upon a plant showing the peculiar growth mechanism of an *Isoetes* stock. The development each season of a starch-packed resting structure, upon which the vegetative apex is born, is analogous to the seasonal production of a corm by such a plant as *Hypoxis* (Iridaceae), with which *Isoetes Drummondii* is found associated in the field. But the analogue must not be pressed too far, for *Hypoxis* has the usual conical apical growing region of most plants and not an invaginated one as in the *Isoetes*. Consequently its stem elongates appreciably in the course of each growing season, while new roots are annually formed adventitiously around the base of the stem. Hence the old corm, composed of exhausted storage parenchyma, becomes crushed below the growing plant each year. But in *Isoetes Drummondii* and other species of the genus root-production is limited to certain lines on the lower surface of the stock, which enlarges each growing season to allow of their expansion. This, coupled with an

¹ Lang, W. H.: loc. cit. Von Mohl, H.: Vermischte Schriften botanischen Inhalts, 1845, pp. 122-8.

invaginated growing apex and the negligible growth in length of the stem each year, forces the exhausted parenchyma outwards, laterally, and so leads in *Isoetes Drummondii* to the development of three lobes corresponding to, and alternating with, the three lines of growth in the root-bearing region.

Roots.

The roots of *Isoetes Drummondii* function only for the few months of each growing season, and then die off. Every year a completely new root system is produced on a new root-bearing surface of the stock (Fig. 7). During the vegetative season these roots are laterally displaced, so that in course of time the roots of any lobe stand in rows forming roughly sectors of concentric circles intersecting the circumference of the stock.

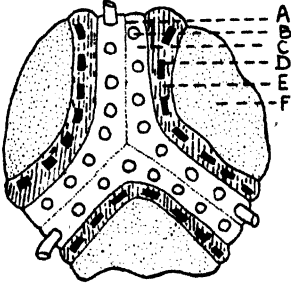


FIG. 7. Diagram to explain state of stock, as seen from below, at close of vegetative season. A, groove; B, roots of current season; C, root-bearing cortex of current season; D, withered roots of previous season; E, root-bearing cortex of previous season; F, scar surface.

Leaves.

The leaves of *Isoetes Drummondii* are produced each year in the following sequence: foliage leaves, megasporophylls, microsporophylls, and the small scale-leaves protecting the apex. The half-dozen or so foliage leaves which are produced first generally die off during the growing season after the sporophylls are expanded. The megasporophylls are more numerous than the microsporophylls, and usually their laminae are the longest leaves formed in the year. Since the microsporophylls are the last leaves produced, and are crushed in the centre of the rosette, their sporangia often show angular compression, while their laminae may be very short.

DESCRIPTION OF THE SPOROPHYLL AND SPORANGIUM.

The lamina of the sporophyll is linear terete, with four large air canals, over which the stomata occur; it has no sclerenchyma strands. The leaf expands below ground into the usual wide membranous wings.

The median portion of the sporophyll base from the region of the ligule to about the lower end of the sporangium becomes tough and cartilaginous towards the close of the vegetative season. This is due to an alteration in the nature of the walls of the epidermis on the abaxial surface, and, near to the ligule, of two or three layers of mesophyll cells in addition. These cells become dark brown and slightly thickened. There is thus formed a structure recalling the leaf-base or phyllopodium of *Isoetes Hystrix* or *I. Duriacii*, but it does not terminate in distal spines nor does it involve the base of the leaf to its insertion on the cortex. It forms a tough shield-shaped structure, in the concave surface of which the sporangium lies.

The cells at the extreme base of the sporophyll of *Isoetes Drummondii* are not sclerized. The mesophyll cells at this region become packed with starch (Fig. 8) during the vegetative season, and towards its close their walls become mucilaginous.

In transverse sections of the leaf taken near the base of the sporangium, it is noticeable that at the initiation of the process there are two centres of

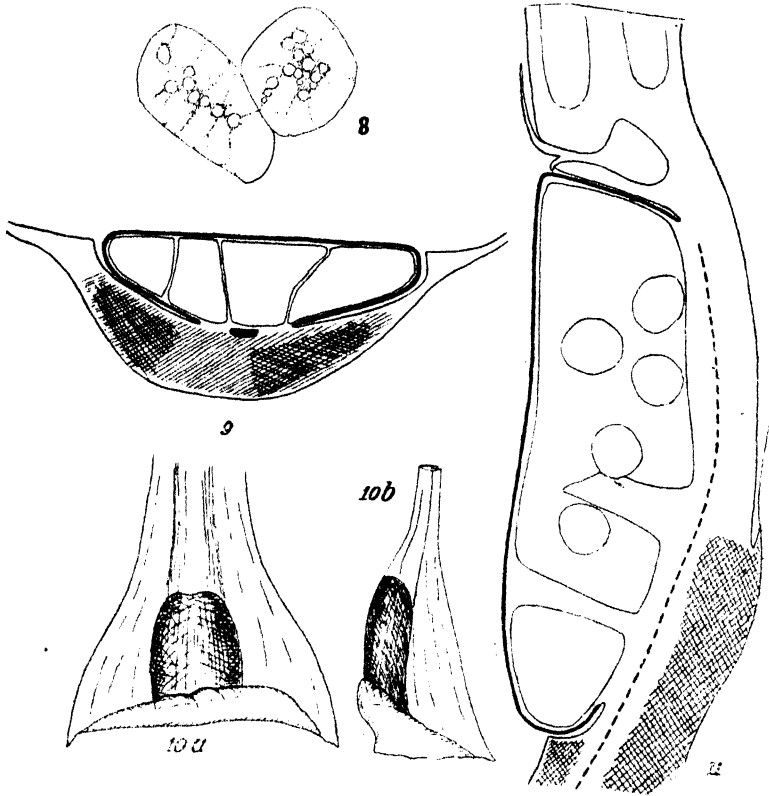


FIG. 8. Cells from mucilage tissue at base of sporophyll collected in October. The walls are already considerably thickened, but some starch remains (cf. Fig. 13). $\times 173$.

FIG. 9. Microsporophyll in transverse section. Starch-containing cells obliquely shaded, two lateral centres of mucilaginous change cross-hatched. $\times 13.5$.

FIGS. 10 *a* and 10 *b*. Megasporophyll viewed abaxially (10 *a*) and laterally (10 *b*), showing pad of mucilage cells extending across base, also extent of tough 'shield' developed behind sporangium. $\times 2.25$.

FIG. 11. Median longitudinal section of megasporophyll collected in October. Cross-hatching shows extent of mucilage tissue. $\times 13.5$.

mucilage formation to be seen (Fig. 9), placed laterally to the leaf-trace. Later these coalesce and a continuous band of mucilage tissue results. Owing to this development there is an increase in thickness at the base of the leaf (Fig. 10), resulting in the formation of a pad or hump of mucilage cells running as a band transversely across the base of the sporophyll, tapering at either end and extending upwards behind the sporangium for

a short distance. The bulk of the mucilage tissue is abaxial to the leaf-trace, though in some cases a little is developed on the adaxial side below the level of the sporangium (Fig. 11). Mucilage also occurs in two or three of the outer cell-layers of the leaf-bearing cortex, but does not extend to any great depth in the stock. Below this sclerenchyma is developed. The process of mucilaginous thickening of the walls appears to go on until drought brings the vegetative activity of the plant to a close.

The sporangia of *Isoetes Drummondii* vary in shape according to their position in the tightly packed rosette of leaf-bases. The outer sporangia are nearly circular in surface view, the inner elliptical, their length being 2-4 times their breadth. In transverse section they have the usual boat-shaped section, i. e. their attachment to the sporophyll is along a relatively narrow strip of tissue. This tissue remains parenchymatous when the sporangium is fully ripe, but the wall of the sporangium undergoes considerable change. At the close of the vegetative season the wall consists of cuticle and epidermis only; the two or three subepidermal layers, which persist in some other species, are lost in *Isoetes Drummondii*. The walls of the epidermal cells become altered as the sporangium matures: they thicken considerably, reducing the lumina of the cells, are dark brown in colour, and probably of a pecto-cellulose nature. The arrangement of the cells of the sporangium wall as seen in surface view (Fig. 12) is significant in connexion with the process of spore dispersal. Those on the flat top of the sporangium are irregular in shape ($50-70 \times 25-28 \mu$) and form a close-fitting mosaic. Those composing the sides are rectangular ($60-100 \times 10-20 \mu$), regularly arranged with their longer walls running vertically. Moreover, in the side-walls of the sporangium certain narrow strands of parenchyma are found, radiating out from the line of attachment to the sporophyll. There may be as many as twenty-four or more such strands around the circumference of a sporangium. These thin-walled cells are obvious lines of weakness in an otherwise tough structure, and, as will be seen below, are functionally important as such in the process by which the spores are liberated.

A further point of importance is the relation of the sporangium wall to the sporophyll. The thickened walls of the sporangium end sharply where they are attached to the parenchyma of the sporophyll, so that the whole way around the base of the sporangium there is a junction of tissues having very different mechanical properties.

PROCESS OF SPORE LIBERATION.

The sporangia and sporophyll bases are in the condition just described at the close of the vegetative season. At its close there follows a period of

four to five months when the plants are invisible below the ground, then at the end of May or in June following the spores are shed.

The first stage in the process of spore dispersal is the separation of the sporophyll bases and sporangia from the subterranean stock and the forcing of them upwards through the soil to the surface. This is effected by the absorption of water in the walls of the mucilage tissue already described. The cell-walls of this tissue absorb water with great avidity, and as a result begin to swell (Fig. 13). Having the stock below them, and the soil around, the line of least resistance is towards the surface of the soil, so

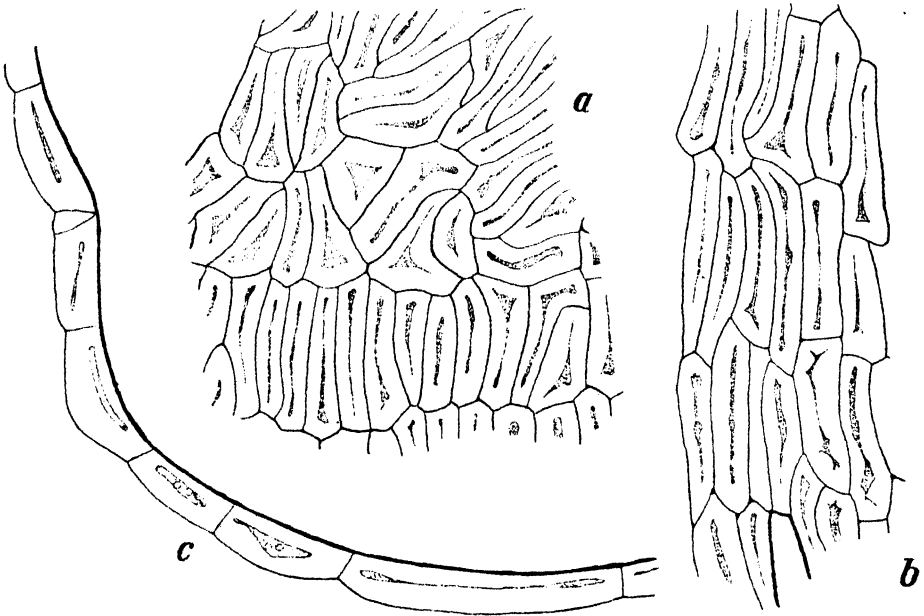


FIG. 12, *a*, *b*, and *c*. *a*, ripe sporangium wall in surface view, showing irregular arrangement of cells on flat upper surface and regular shape of cells of side-walls (at bottom of figure). *b*, cells in side-wall of ripe sporangium. *c*, transverse section of ripe sporangium wall, showing cells somewhat distended with water and stout cuticle. Camera lucida outlines. $\times 93$.

that, expanding in this direction, they force the imbricate mass of sporophyll bases upwards until it reaches the surface (Fig. 3).

The second stage depends upon the continued presence of an excess of moisture. Sooner or later the mass of sporophylls becomes sodden and falls apart (Figs. 14 *a* and 14 *b*). It is only now, when the walls of the sporangium are thoroughly saturated, that the liberation of the spores occurs. The thick-walled cells of the sporangium wall have absorbed moisture and tend to expand, but, being bounded externally by a cuticle which is less expansive than the cell-walls, the tension set up is such as to produce an eversion of the sporangium. First the wall ruptures at any point on its circumference. One or more segments of the tough wall tear away from

their attachment to the parenchyma of the sporophyll, and from each other along the lines of weakness already noticed in the side of the wall. They rapidly roll upwards, inside out. Once the process of tearing away has begun it proceeds very quickly, and in a few seconds the whole sporangium wall has rolled back upon itself in a tight coil. Some of the spores are carried back with it, but the main mass is left *in situ* in the depression at the base of the sporophyll. Great numbers of megaspores and microspores are thus set free upon the break up of a sporophyll mass within a few square centimetres.

The opening of the sporangia has been followed many times in the laboratory by bringing ripe dry sporophylls there and allowing them to absorb moisture. That the process depends upon differences in tension

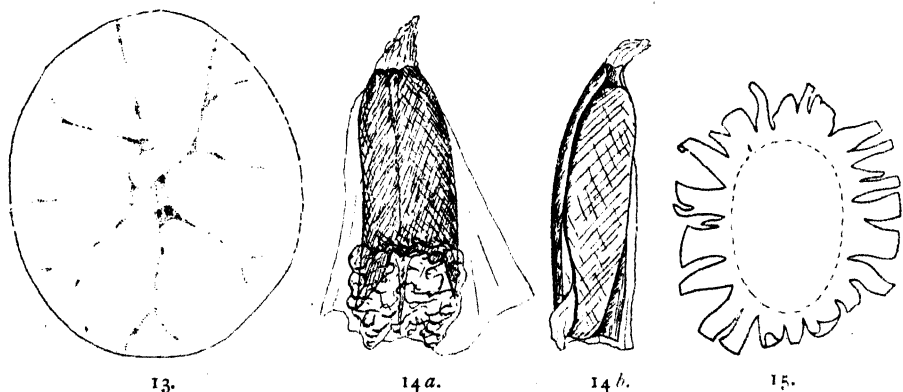


FIG. 13. Single cell of mucilage tissue, with walls fully extended with water. From fresh material collected in June at time of the sporophylls being elevated. $\times 173$.

FIGS. 14 *a* and 14 *b*. Single microsporophyll removed from dry mass of bases and sporangia found on surface of soil in June. $\times 3$. 14 *a* shows abaxial view; at top of shield withered remains of lamina is seen, at base an irregular lump of dry mucilage. 14 *b* shows adaxial surface, the elongate flat-topped sporangium still in place.

FIG. 15. Sporangium wall in surface view, after it has torn away from sporophyll, showing fimbriated segments into which side of wall has torn.

between the inner and outer surfaces of the wall consequent upon the absorption of water can be shown by placing the freed, rolled-up sporangium wall in different strengths of a dehydrating solution such as alcohol. A solution of 70–80 per cent. causes the wall to unroll and float in the liquid like a membrane (Fig. 15), roughly star-shaped, with truncated points. Stronger alcohol causes the membrane to close back into its original position before tearing open. The same sporangium wall can be made to repeat the process several times by varying the proportions of alcohol and water in which it is placed.

CONCLUSION.

It is not the purpose of this paper to enter into a general discussion of the morphology of *Isoetes*. So far as the stock is concerned, the chief

point of interest that an examination of *Isoetes Drummondii* has brought out is that, as in other species of the genus, there is a regular annual production of sets of leaves and roots upon a special cortex developed to allow of their expansion. There is a close correlation between the growth of leaf- and root-bearing surfaces which finds its expression in the development of three lobes and three clefts in the leaf-bearing cortex, to correspond with the three grooves from which the roots appear. The climatic conditions under which *Isoetes Drummondii* grows, resulting in a regular alternation of vegetating and resting seasons, makes the annual production of roots and leaves unusually definite.

Before a general discussion of the sporangial mechanism of the genus could be attempted much further information, derived from a study of various species in the field and with abundance of fresh material, is desirable. A further study of such species as *I. Hystrix* and *I. Duriaei* in the Mediterranean and of *I. Butleri* and other species growing in damp soil in the United States would be particularly interesting for comparative purposes. Whether any of these species would show a spore-dispersal mechanism comparable to that of *I. Drummondii* it is impossible to say. In this connexion it is interesting to recall the presence of cells with mucilaginous walls in the sporophyll of *I. Hystrix*.¹ In this species the mucilage tissue is distributed in two strands lateral to the sporangia; the function of these strands is at present uncertain. In whatever way the spores of *I. Hystrix* are freed, the mechanism of dispersal must be efficient. Durieu² describes 'un gazon fin et uniforme' covering certain hill-tops in Algiers, which he found to be composed of *Isoetes*, though at first the plants were mistaken for a grass. In South Australia it is easy to mistake the rosettes of *I. Drummondii* for those of some phanerogam.

The vast majority of the Pteridophyta free their spores under dry conditions, the familiar mechanism of the annulus depending on progressive desiccation for its action. The liberation of the spores in the subaquatic species of *Isoetes* appears to depend upon a process of decay. In *Isoetes Drummondii* there is a special mechanism for freeing the spores which depends upon saturation with water, not on dryness, for its action.³ Many of the other peculiar features of *Isoetes Drummondii* described above appear to be in the nature of preparations for this remarkable method of spore dispersal.

¹ Hill, T. G.: Ann. Bot., xx. 267-73, 1906.

² Quoted by Motelay, L., and Vendryès: Reprint from Actes de la Soc. Linn. de Bordeaux, p. 95, 1884.

³ As it stands, the case of *Isoetes Drummondii* would appear to be unique among the Pteridophyta. Such xerophytic developments as the sporocarps of *Marsilia* and *Pilularia* afford only remote analogues.

SUMMARY.

1. *Isoetes Drummondii* is a plant widely distributed in certain parts of South Australia, where it grows terrestrially in seasonal swamps during the period of winter rainfall. During the dry summer it aestivates, as do the other geophytes with which it is associated. •

2. The stock is buried to a depth of about 2 cm., and during the vegetative season only a small rosette of linear leaves is visible above the soil.

3. The stock is trilobed, the projecting portion of each lobe being built up of a number of segments or caps, the caps being the whole of the leaf- and root-bearing portions of the stock developed in previous growing seasons. The abscission of such caps is a result of the regular alternation of growing and resting periods (during which there is great desiccation) in the life-history of a plant having the growth mechanism of an *Isoetes*.

4. On the approach of the dry season the leaves dry up and become detached, leaving their tough bases and sporangia *in situ* upon the stock, wholly buried and invisible.

5. Early in the rainy season following, the hardened bases of the sporophylls are forced above the surface of the soil in a projectile-like mass, carrying with them the sporangia, by the expansion of certain pads of mucilage cells formed at the close of the previous vegetative season on the extreme bases of the sporophylls and from the superficial cells of the leaf-bearing cortex. About the same time the leaves of the new vegetative season begin to appear.

6. The imbricate mass of sporophyll bases breaks up upon the surface of the soil, and the spores are set free by a tearing away of the sporangium wall from its attachment to the sporophyll when sodden. This is due to a difference between the tension of the inner and outer surfaces of the sporangium wall when saturated, and results in an eversion of the wall.

Observations in Malaya on Bud-rot of Coco-nuts.

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With Plates I-VII.

JOHNSTONE'S work on Bud-rot of Coco-nuts (4) in the West Indies was the first definite contribution towards establishing a probable cause of the disease. Coleman (3) investigated a disease of the Areca Palm (*Areca Catechu*), and proved that *Phytophthora omnivora* var. *Arecae* (Coleman) could attack this palm at the crown. Butler (2) about the same time worked on a Bud-rot of Palmyra Palm (*Borassus flabellifera*), and recorded successful inoculations of the young central heart leaves with *Pythium palmivorum* (Butler); recently Butler has reconsidered this fungus as *Phytophthora palmivora* (Butler). Johnstone (loc. cit.) forwards evidence for a bacterial origin of Bud-rot in *Cocos nucifera*, the causal organism being regarded as *Bacillus coli* (Escherlich) Migula. There would appear then to be little in common between the diseases of the three palms mentioned, except similar symptoms produced by very different causes.

Shaw and Sundaraman (8) working on Bud-rot of Coco-nuts in Malabar performed inoculation experiments and stated that *Phytophthora* (*Pythium*) *palmivora* (Butler) was the cause of the disease. Their observations have received much support from the recent work of Reinking in the Philippines and Ashby in Jamaica. Ashby (1) considers *Phytophthora palmivora* (Butler) as the cause of Coco-nut Bud-rot in Jamaica. Reinking (7) says, 'From these researches it can be stated with certainty that *Phytophthora faberi* (Maubl.) causes Coco-nut Bud-rot; bacteria are apparently in the majority of cases always secondary, but are concerned with destroying the weakened tissues'. These recent investigations bring the causes of the palm Bud-rots, mentioned above, more into line; all are caused by different species of the genus *Phytophthora*.

No definite information, from Malaya or other rubber-growing countries of the Middle East, on the *Phytophthoras* causing diseases of Coco-nut

palms has yet been recorded. The matter is one of some urgency as *Phytophthora faberi* (Maubl.) has long been known to cause a serious bark disease of *Hevea brasiliensis*. The absence or presence of different host plants is of primary importance when considering control measures for serious plant diseases. For this reason, following on a visit from Professor O. A. Reinking, some attention has been given to Coco-nut Bud-rot during the last twelve months, though this disease never has caused, in the writer's experience, any serious loss in Malaya.

SYMPTOMS.

The experimental results to be described are valid only in so far as an accurate knowledge of the symptoms, both early and advanced, can be obtained. For this purpose, it is necessary to go into considerable detail, drawing upon the experience of investigators in all countries where Bud-rot is a disease of importance. Johnstone (4) gives a general diagnosis of the disease as follows: 'The common name of the disease, Bud-rot, well describes its nature, for in its acute or advanced stages the bud of the tree, i. e. the growing-point in the centre of the crown, is affected by a vile-smelling soft-rot which destroys all the younger tissues. At this stage most of the nuts have fallen, the lower leaves are turning yellow, and the middle folded and undeveloped leaves are dead, and hang down between the still green surrounding leaves. Signs of the disease in its incipency are (1) the falling of the immature nuts; (2) a staining of the opening flower spikes, partly or wholly, of a rich chocolate brown; and (3) the drying and bending over of the middle undeveloped leaves.' The same author also gives the symptoms (*ibid.*) as observed in different countries. On page 12: 'In many cases the descriptions (from the West Indies) are so meagre that it is impossible to identify them with the Bud-rot; nevertheless the one characteristic, the rot in the heart-tissues, is believed to apply only to this disease. In addition, the dying of the central undeveloped leaves is taken as a sign of the Bud-rot.' On page 19, under Philippine Islands, he quotes: 'As soon as the youngest leaf is noticeably discoloured it can easily be drawn out.' On page 10, under Ceylon, Petch is quoted: 'The first indication of the disease in the case of young plants is the withering of the youngest unfolding leaf. This turns brown and can be pulled out of its sheath; it is then found to end in a soft brown mass.' On page 20, under German East Africa: 'Soon after the first appearance of the disease the heart leaves can be drawn out, as the bottom is rotted off.' Shaw and Sundaraman (8) say: 'The first symptom of which a diseased tree may be recognized is that the central leaf turns brown, collapses, and dies.' They also quote (*ibid.*) that Petch in Ceylon lays stress on the early withering of the central leaf in young trees, a point

which up till then had not been mentioned by West Indian observers. Reinking (7) says: 'The first symptom is a withering of the youngest unfolded leaf, followed by the leaf turning brown. Gradually the next younger leaves wither and turn brown, until the entire central group is affected. At this stage the central leaves may be easily pulled out. Frequently in advanced cases, they fall over.' Ashby (1) says: 'The existence of the disease is indicated by the pale colour, bending over, browning, and breaking down of the heart leaf.'

The quotations from the numerous investigators in both hemispheres are in agreement as to the general symptoms of Bud-rot in Coco-nuts. Investigators in the West Indies, previous to Johnstone, confused other symptoms with those of Bud-rot; the commonest symptom mistakenly associated with Bud-rot being the 'red-ring' in the stem, which has since been proved by Nowell (5) to be caused by a nematode attack. Therefore we have a definite guiding line as to the interpretation of any experimental inoculation results: 'The initial browning and death of the heart leaves, which may fall over or can easily be pulled out; if the bud-tissue is now examined it will presumably show the rot which typifies the disease.' It may be noted here that Johnstone (4) appears to be the only investigator who stresses the rotting of the central tissues. Ashby (1) also remarks that trees successfully inoculated with *Phytophthora palmivora* (Butler), i. e. 'showing rows of depressed spots in the central leaves', do not necessarily die at the heart.

OBSERVATIONS IN THE FEDERATED MALAY STATES.

Dating from the time of Professor Reinking's visit to the Federated Malay States, the senior author has been trying to obtain field information on Bud-rot of Coco-nuts, intending to prosecute an intensive investigation into the cause of the disease. A *Phytophthora* disease, causing falling of nuts, has been noted in Ceylon by Petch (6), but no evidence of connexion with Bud-rot is offered. It is interesting to note that Johnstone (4) mentions, as one of the incipient signs of Bud-rot, the falling of the immature nuts. This feature has been commonly met with in Malaya since 1914, but it can be categorically stated that, in this country, the falling of immature nuts has little or no connexion with Bud-rot of Coco-nuts. Ashby (1) describes a form of Bud-rot, the earliest symptom of which is usually the dropping of young nuts from one or more spikes, which blacken and wither up.

This disease has never assumed epidemic form except in one small instance, to be detailed below, in Malayan plantations. Most Coco-nut estates find a few cases, usually widely separated, at various periods of the year. Beyond cutting the diseased palms and burning them, nothing is done, and from year to year there has been no cause for uneasiness with

regard to spread. This might be considered unusual in view of the general prevalence of 'Black Stripe' caused by *Phytophthora faberi* (Maubl.) on the rubber plantations of Malaya; one can only assume that the general conditions in Malaya are somewhat inimical to the well-being of this fungus, an assumption which any experienced observer in Malaya would not countenance.

During 1920 an urgent call was received from an estate in the chief Coco-nut centre in Malaya, asking for immediate advice regarding an outbreak of Bud-rot. A visit was made by the senior author, who found a three-acre field with practically every tree showing signs of Bud-rot, some in a very advanced state of decay. This field was surrounded by fields containing trees of similar age, well grown, and with no signs of Bud-rot. The badly affected field was inundated twice a day by tidal water, and the trees were very backward in consequence. There was little doubt that the primary cause of the trouble in this case was the daily inundations.

ISOLATION AND INVESTIGATION OF DISEASED TISSUES.

A large number of specimens in all stages of decay were taken to the laboratory from the estate mentioned above, and isolations made from the advancing margin of the diseased tissue. As the central leaves were all destroyed it was not possible to search for the depressed rows of spots described by Shaw and Sundaraman (8) and later by Ashby (1). The specimens of bud-tissue used to obtain isolations from were put up in spirit and sectioned.

One specimen (Plate I, Fig. 1) showed the rot extending below the bud to a depth of three to four inches. Isolations and sections were made from this material, and were substantially the same as the ordinary specimens (Plate I, Figs. 2 and 3).

Examination of sections showed no obvious fungal hyphae in any of the specimens. The isolation resulted in three different organisms, one producing a deep-red pigment, the second a pale lemon-coloured growth in culture, and the third a pure-white growth.

Preliminary examination suggested all three as bacterial cultures. They were finally sent to Dr. Fletcher, Bacteriologist to the Institute of Medical Research, Kuala Lumpur, who reported as follows: 'All are non-motile. The organism producing the red pigment is a minute Gram-positive bacillus'—this was taken at the commencement to be *Bacillus prodigiosus*.

'The organism which gives the pale lemon-coloured growth is a small Gram-positive bacillus, possibly *B. flavo-coriaceus* (Eisenberg).

'The white growth is a minute Gram-positive fungus.' The senior author on careful examination would place this in *Sarcinomyces*.

A characteristic feature in Malaya, in advanced cases of Bud-rot, is an obvious pinkish discoloration, due probably to the presence of the red bacterium in considerable quantity. This organism grows well on meat-extract agar; the *Sarcinomyces* (?) develops well on green-pea agar; the lemon-coloured bacterium was difficult to grow, the best results being obtained on potato-mush agar. †

LABORATORY TESTS.

After Professor Reinking's visit the senior author carried out various parallel tests on Coco-nut bud-tissue with the above-mentioned bacteria and *Phytophthora faberi* (Maubl.) isolated from 'Black Stripe' disease of rubber bark. For this purpose Coco-nut cabbage tissue was obtained and inoculated with the various organisms. These test pieces were placed under bell-jars and similar inoculated pieces were kept as controls. The results were extremely variable, as might be expected, but these tests undoubtedly showed that the bud-tissue was a highly favourable medium for all the organisms concerned. The control test pieces remained sound for several days after the inoculated ones were rotten.

The behaviour of these organisms led to the consideration of the experimental proof of the cause of Bud-rot. Obviously, the cabbage being formed of tissue containing abundant nutritive material, there would be some danger in basing conclusions on results obtained by direct wounding of the bud-tissue, no matter how small the puncture made. A fair simile would be the difference between inoculating any artificial culture medium with inoculum from a culture, and inserting a sterile needle in a tube of the same medium. Analysing Reinking's (7) latest results on this basis we find :

		Inoculated.		Controls.	
		+	-	+	-
Young trees.	(1) Stab in damp chambers	3	2	0	0
"	(2) Stab outside in shade	14	1	0	7
"	(3) Uninjured in shade	2	6	0	2
Old trees.	(4) Stab (three mature trees outside, one not examined)	1	0	1	0

These results were obtained by using *Phytophthora faberi* isolated from Cacao pod-rot. Later he isolated *Phytophthora faberi* from the woody tissue below the growing-point of a Coco-nut palm showing Bud-rot. With this fungus thirteen inoculations were made in seedling Coco-nuts by stabs—all were successful. Only two controls were kept which remained healthy.

* On the above reasoning and analysis the present writers were of the opinion that more work was necessary before accepting Reinking's conclusion *in toto*, i.e. 'From these researches it can be stated with certainty that *Phytophthora faberi* (Maubl.) causes Coco-nut Bud-rot; bacteria are

apparently in the majority of cases always secondary, but are concerned with destroying the weakened tissues'.

Again, the writers are convinced that too much importance is attached to results of inoculation on seedlings in the laboratory. No disease on mature trees can be considered finally proved until the typical symptoms have been reproduced by artificial inoculations on mature trees. Valuable information as to the possible cause of the disease on mature trees may be obtained by experimental inoculations on seedlings, but more often than not this is put forward and is often accepted as proof conclusive. That such is not always the case the following series of experimental inoculations will show very clearly.

As we were not attempting to prove a specific cause of Bud-rot no attempt was made to identify the cultures worked with specifically. It will be convenient for our purpose to tabulate results under group letters, the following key giving the necessary information to the organism used for inoculating the trees:

Organism *A* = *Phytophthora faberi* (Maubl.).

„ *B* = Red-pigmented bacillus.

„ *C* = *Sarcimonys* sp.

„ *D* = Mixture of *B* and *C*.

The inoculations were made by clearing away the leaves and leaf bases on one side of the tree. A suitable point for inserting a small gouge was chosen and cleaned externally with spirit; the instrument was flamed before boring into the heart. When the tender central tissues are reached there is usually some exudation of water; if the gouge is now withdrawn carefully the tissue from the bore-hole will come out lying in the concave side of the gouge. This tissue was inoculated with the various organisms and pushed back into the bore-hole with the handle of a flamed needle-holder.

The tabulated results show that in the Group *A* inoculations only one tree, No. 4, remained healthy up to 8.3.21. Tree No. 7, badly attacked on 8.10.20 and 9.11.20, is fully recovered on 8.3.21. Trees Nos. 8, 9, and 10, attacked at various stages, show no definite signs on 8.3.21, but on this date eight trees still show signs of attack in various stages.

Of the six control trees for Group *A*, two were attacked by beetles, which somewhat upsets the value of the control. However, three trees appeared to have a bad attack on 8.10.20, but on 8.3.21 only one tree out of five (one was cut out for examination) showed signs of attack.

The trees in Group *B* on 8.10.20 showed a rapid and very definite attack, seven trees out of eight being very severely attacked. At this date, if the red-pigmented bacillus had been the only organism worked with, we might have concluded that this was the probable cause of the disease in Malaya. All the central shoots were black and decayed, most of them

TABLE I.

Date of Inoculation.	Date of Examination.	Group.	Remarks.
30.8.20	8.10.20	A	⊕ = cut out on 7.3.21
do.	9.10.20	"	⊕ = attacked by beetle
do.	8.3.21	"	⊕ = cut out on 8.10.20
30.8.20	8.10.20	Controls for Group A	
do.	9.11.20	"	
do.	8.3.21	"	
1.9.20	8.10.20	B	
do.	9.11.20	"	
do.	8.3.21	"	
1.9.20	8.10.20	C	
do.	9.11.20	"	
do.	8.3.21	"	
1.9.20	8.10.20	D	
do.	9.11.20	"	
do.	8.3.21	"	
1.9.20	8.10.20	Controls, Groups B, C and D	
do.	9.11.20	"	
do.	8.3.21	"	

⊕ = Badly attacked, with central leaf falling or easily pulled out.
 Δ = Badly attacked, black and mouldy, central leaf stiff and not easily pulled out.
 ⊞ = Mild attack, central shoot slightly mouldy, and usually recovering quickly.
 ⊖ = No result.

falling over on their own. However, the position is very different on 8.3.21, when these trees appeared to have recovered to some extent. The manner of recovery will be discussed below.

The trees in Group *C* behaved in much the same manner as those in Group *B*, though the initial severity of attack was not so noticeable. During the experimental period five out of six trees were attacked, but on 8.3.21 the five trees still remaining were quite healthy.

Group *D*, inoculated with a mixture of *B* and *C*, shows a different course of events, the attack being slowed up considerably. On 8.3.21, of the five trees remaining, all showed evidence of attack. This result is probably connected with the initial hold-up; it is to be expected that all these trees will recover.

The twelve controls established for the bacterial infections show definitely that without inoculating material the infections are neither so rapid nor severe. It is to be expected that the gouge going through the leaf-bases will, in some cases, carry material into the centre which, on reaching the nutritive bud-tissue, will ultimately develop and possibly cause a Bud-rot. In these twelve controls only two showed definitely on 8.10.20, with one mild attack. On 9.11.20 another tree was badly attacked, and another very mildly attacked, and one of the previous badly attacked trees was recovering. On 8.3.21 only one showed a bad attack with three mild attacks.

The results brought forward undoubtedly proved the point we set out to establish, i. e. 'that no conclusions based on "stab inoculations" can be considered satisfactory, when the inoculated tissues are rich in easily converted food materials'.

This point is of the utmost importance in a consideration of Bud-rot of palms, for many investigators working on this problem have based their conclusions largely upon results obtained by 'stab inoculations'. In order to establish the point more firmly, further inoculations were made with three different organisms commonly appearing on rotting bud-tissue in the laboratory. These inoculations were performed some two months after the first set, so that any change of conditions might be expected to influence these later results. The groups follow on in order:

- Group *E* inoculated with *Thielavia* sp.
- " *F* " " *Mucor* sp.
- " *G* " " *Bacillus flavo-coriaceus*.

The results of the later experiment are entirely concordant with the previous one. The Group *E* inoculations went off quickly, four trees being badly attacked, but on 8.3.21 all except one were recovering—one remained healthy throughout. The sequence of events parallels the Group *B* inoculations. The Group *E* inoculations started very slowly

TABLE II.

<i>Date of Inoculation.</i>	<i>Date of Examination.</i>	<i>Grew.</i>									<i>Remarks.</i>
8.10.20	9.11.20	E	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	
do.	8.3.21	"	Δ	Δ	Δ	⊕	⊕	⊕	⊕	⊕	
8.10.20	9.11.20	F	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	
do.	8.3.21	"	⊕	⊕	⊕	⊕	Δ	⊕	⊕	⊕	
8.10.20	9.11.20	G	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	
do.	8.3.21	"	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	
8.10.20	9.11.20	Controls for E, F, and G	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	○ = cut out on 7.3.21
do.	8.3.21	"	Δ	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕ = beetle attack
do.	8.3.21	Inter relation of signs as in Table I.	Δ	⊕	⊕	⊕	⊕	⊕	⊕	⊕	

and on 9.11.20 the trees appeared quite healthy. The second inspection on 8.3.21 revealed four very badly and one mildly attacked and one healthy. The further course of this group will be watched. The sequence is similar to that observed in the mixed Group *D* inoculations.

The Group *G* inoculations showed three badly infected cases on 9.11.20, but on 8.3.21 only one remained in a badly diseased condition, while three others which had suffered were well on the road to recovery; only one was healthy throughout, and one was cut out for examination.

The controls were again influenced by beetle attack, noted previously in the Group *A* controls. On 9.11.20 there were only three mild attacks and three attacked by beetles; these latter were otherwise healthy. On 8.3.21 there was only one badly diseased tree, three slightly attacked were almost recovered, and one still showed a mild attack with no obvious attempt at recovery.

INTERPRETATION OF RESULTS.

The essential point in interpreting the foregoing results is the recognition of what is to be considered a successful inoculation. The writers have stated their guiding line (*supra*), drawing upon the experience of previous investigators. Johnstone (4) has further remarks upon the difficulty of inoculating the heart of a Coco-nut tree. He says, 'Inoculations made below the heart fail to produce the rot, since these tissues naturally soon harden as a part of the mature trees. If, on the other hand, the inoculation be made above the heart, amid the growing leaves, their extremely rapid elongation takes the inoculation point out of the surrounding soft tissues. The inoculated tissues then become green and membranous, and thus resist the advance of the rot.'

Johnstone (4), when considering inoculation experiments, rightly stresses the necessity for the actual rot of the bud-tissue. As far as our acquaintance with the literature goes other investigators have been satisfied to produce a rotting and falling over of the central heart leaves—in such case our inoculations marked $\oplus$ could not be misinterpreted.

The plates show some of our typical results. Plate II shows a tree from the Group *G* inoculations, the central leaves leaning and about to fall. Plate III shows the central leaves fallen out of a tree in Group *C*. Plate IV shows one of the control trees which became infected with the centre falling out. Plate V shows a similar effect after a *Phytophthora faberi* inoculation (Group *A*), and Plate VI shows another tree inoculated with the same fungus. These photographs can leave no possible doubt as to our success in obtaining what are usually considered definite signs of Bud-rot.

As indicated in the tables, trees from the various groups were taken out at intervals to note the progress of the inoculations. On 8.10.20 two trees

from which the central shoots had fallen out were cut down and examined : these were No. 1 in Group *D*, and No. 6 in the controls for Group *A*. The first showed a mixed crowd of putrefying organisms on the top of the bud-tissues and investing this externally for $1\frac{1}{2}$ in. all round, the pink colour, probably due to the red-pigmented bacillus, predominating. When the heart-tissue was cut open there was no penetration to be observed : the cabbage appeared quite sound internally. The second tree (Group *A* control) showed the bud invested similarly, but, on cutting open the heart-tissue, penetration to a depth of 2 in. internally was found. This was undoubtedly Bud-rot.

The observations made on these two trees led to the conclusion that in most cases where the central leaves were badly diseased, a rotting of the bud would follow, and, as comparatively few of the controls showed a bad attack, the writers felt justified in assuming at the time that the introduced organisms, placed directly in the bud, were capable of causing, in the field, a typical Bud-rot on mature trees.

The attacked trees were left to develop, but, as time passed, it became obvious that events were pursuing a course contrary to that anticipated. This was most obvious in Group *B*; four of these had their central shoot pulled out quite easily, the others were left untouched. On 8.3.21, however, all had recovered.

The general method of recovery is distinctly peculiar. Plate VII, Fig. 1, shows the type. The central shoot has disappeared, but from the side of the bud below the remains of the central shoot a lateral shoot is pushed out. The leaves comprising this lateral shoot are strangely aborted, the leaflets being very stiff and only partially developed. Growth of this lateral shoot continues, and it takes the place of the central shoot. The leaves open out and present the appearance shown in Plate VII, Fig. 2, which is a photograph of tree No. 1 in Group *C*.

This tree was cut out on 7.3.21, and a close examination of the central leaves and bud-tissue made. Another central shoot was coming up, and on examination this showed the same aborted leaves inside the central sheath. The bud was healthy, as were the lower 18 in. of the new central shoot. Above the lower 18 in., for 12 in. or so, undoubted evidence of a diseased condition was observed—a black, slimy bacterial appearance being quite marked. A photograph of this peculiar state of affairs was a failure. Another tree was cut out which showed just the same condition.

On 7.3.21 tree No. 2 of the *Phytophthora faberi* group was cut out for examination. Before cutting, this tree had only the external leaves standing (Plate VII, Fig. 3), the bud-tissue being absolutely rotted (Plate VII, Fig. 4). This has been the only definite case of Bud-rot noted throughout all our inoculations.

CONCLUSIONS DRAWN FROM WORK RECORDED ABOVE.

It can be taken as definitely established :

1. That if the nutritive bud-tissue of Coco-nuts is a suitable pabulum for any saprophytic organism, either bacterium or fungus, this will develop and cause symptoms usually associated with Bud-rot if inoculated directly into the bud-tissues.

2. Owing to a very definite resistance exercised by the bud-tissues of mature trees against infection, such organisms in the absence of suitable conditions will not develop beyond a certain stage, marked by the death of the central shoot. If the central shoot dies, and the bud is invested externally with the invading organism, the bud-tissues have the power of pushing out a lateral, by means of which growth is continued to take the place of the diseased central shoot.

Bearing of above on previous Bud-rot investigations.

The above conclusions exercise considerable influence on previous recorded work. The prevailing idea that growth is no longer possible if the central shoot is killed must now be considered a fallacy, though it must be admitted that healthy growth is not immediate even if lateral shoots are produced. There is no doubt, however, that the trees producing the aborted central shoots do finally recover and put out healthy ones.

The proved resistance of mature trees negatives conclusions based on seedling inoculations, more especially when these inoculations have been aided by artificial humidity conditions as in damp chambers. Short-time observations in recording results of inoculation experiments on mature trees must also, in the light of our experiments, be of minor importance when the question of experimental proof of Bud-rot is considered.

Whilst concerned primarily with Reinking's work (7), it will be advisable to consider the work of other authors in view of the recorded facts. Coleman (3), in his work on the Areca Palm, showed his *Phytophthora omnivora* var. *Arecae* to be primarily concerned in causing a nut disease, but he records one inoculation made in the top of the tree with a suspension of zoospores, which, two weeks after inoculation, showed the fungus grown through the underlying leaf-sheaths and attacking the growing-point. He concludes from this that a direct infection of the tree-top (presumably bud-tissue) by means of zoospores is possible, a perfectly admissible conclusion, even from one observation only.

Butler (2), in his work on Palmyra Palm Bud-rot, got undoubted parasitic penetration of the leaf sheaths with *Pythium* (*Phytophthora*) *palmivorum* (Butler), but twenty-eight days was the longest time allowed between inoculation and examination. No rotting of the bud was observed in any single case.

Johnstone (4) in his work, records his inoculations, and worked more

along the lines of the present writers, by trying to induce rotting of the bud-tissues on mature trees by artificial stab inoculations. His conclusions are based on results in which the time period allowed between inoculation and examination was generally from eight to sixteen days. He also records two other inoculations: one performed on June 22, with a bacterial culture, showed a Bud-rot on October 21; the second, similarly treated on July 22, showed no evidence of Bud-rot on August 6. As stated, too much importance cannot be attached to results and conclusions when the period elapsing between inoculation and examination is so short, for it is obvious from the inoculations recorded above that, even if organisms are introduced directly into the bud-tissues, their behaviour may be very different. The Group *D* and Group *F* inoculations were significant in this respect, for the period elapsing between inoculation and the first signs of diseased tissues was well over two months, whereas in all the other groups the first signs were almost immediate.

Shaw and Sundaraman (8), working with Butler's *Pythium* (*Phytophthora*) *palmivorum*, when considering Bud-rot of Coco-nuts, obtained results similar to those of Butler in his work on Palmyra Palm Bud-rot. They obtained depressed spots without wounding on the tender central leaves, indicating a parasitic penetration by the fungus. They also obtained collapse of the central shoot in a seedling Coco-nut when inoculated and kept in a damp chamber.

Their inoculation experiments were wholly upon seedlings, no mature palms being utilized. The only admissible conclusion here as in the previous work of Butler, is that inoculations with *Phytophthora palmivora* (Butler) result in a decided parasitic penetration of the central leaves, without any necessary connexion with rotting of the 'Bud'.

Reinking's work has already been considered. In view of the fact that his inoculations were practically all stab inoculation on seedling Coco-nuts, the evidence brought forward for *Phytophthora faberi* as the cause of Bud-rot in the Philippines cannot be considered satisfactory, and no definite conclusion as to the cause of Bud-rot can be arrived at. As regards our *Phytophthora faberi* (Maubl.) inoculations, they were no better or worse than inoculations made with the other recorded organisms. Most are recovering, and, although the only genuine Bud-rot occurred in this group, a successful inoculation on one tree is not sufficient base important conclusions upon, when the remainder behave as other groups inoculated by widely different organisms.

Ashby's work (1) is of interest as confirming Butler's (2) and Shaw and Sundaraman's work (8). He records similar features, and from his observations fairly concludes that inoculated trees showing rows of typical *Phytophthora palmivora* spots on central leaves do not necessarily die at the heart.

CONCLUSIONS.

The general impression obtained from the results of the workers cited is that all the Bud-rot diseases of Eastern and Western Hemispheres are attributable to one or more species of *Phytophthora*, but, judging from our experiments, it would appear that what has been proved is that *Phytophthora palmivora* (Butler) functions as an obligate parasite on the tender central leaves of most palms, but has not been proved to cause rotting of the 'heart-tissues'. This obligate parasitism has been very definitely demonstrated in India by Butler (2) and Shaw and Sundaraman (8), and lately by Ashby (1) in the West Indies. However, this cannot be regarded as any proof of the cause of the rotting of the bud-tissues, no more than the death and falling of the central leaves in our experiments can be considered as proving the rotting of the central bud. In these latter cases, if the bud was rotted, then the conception that the slightest invasion of the heart-tissues by a parasitic organism will result in death must go by the board.

The question then arises, 'What is Bud-rot?' The conception of Bud-rot causing death of the tree must be strictly limited to the rotting of the heart-tissues; a diseased condition of the central leaves does not necessarily connote the death of the tree.

Our conclusions are well stated in a review signed 'W. N.' in the 'West Indian Agricultural News'.¹ The writer reviews Reinking's recent work (7), and says: 'Notices of Mr. Reinking's paper have already appeared in the Journal of the Agricultural Society of Trinidad and of the Board of Agriculture in British Guiana, and the announcement has naturally aroused the greatest interest, from its possible bearing on the Bud-rot problems in the West Indies.

'The present reviewer, when recently in Trinidad, found a tendency on the part of Coco-nut planters to assume that the results obtained in the Philippines were immediately applicable to the local affection, while the British Guiana Journal in an editorial comment states: "There is little doubt that a careful scientific investigation here will prove a similar relationship between *Phytophthora faberi* and Bud-rot."

'Assumptions of this kind are to be deprecated, and there are special reasons for caution in the case of Bud-rot. The writer (W. N.) has insisted from time to time on recognition of the fact that the existence of Bud-rot in coconut palms is not of itself evidence of the presence of a specific disease, or of disease at all, in the ordinary sense of the word. Bud-rot is a condition which may be induced by mechanical and chemical or parasitic interference with the life processes of the palm. The material of the heart is extremely tender, and when the natural resistance² of the living tissue

¹ Vol. xviii, No. 461, December 27, 1919.

² Possibly greater than is usually thought.—A. S. and J. L.

is reduced, it forms a highly nutritive medium suitable for the rapid development of any of a large variety of possible invading organisms. In the case of epidemic or infective Bud-rot, the issue is narrowed down to the responsibility of a transferable parasite; but there is no ground for assuming that the parasite concerned in producing a condition of such a general nature is necessarily or even probably the same in different situations. J. R. Johnstone, now confirmed to some extent by Reinking, has put forward evidence to show that *Bacillus coli* may be effective in setting up Coco-nut Bud-rot. The widely prevalent and destructive Bud-rot of palms in Southern India has long been known to be due to *Phytophthora palmivora* (*Pythium palmivorum*), and the same fungus has recently been found by S. F. Ashby in connexion with Coco-nut Bud-rot in Jamaica.'

This statement from the West Indies is of considerable importance in view of the facts put forward in this paper. The views of the present writers are faithfully stated in the above quotation, although not agreeing that Bud-rot of palms in India has been proved to be due to anything. However, *Phytophthora palmivora* (Butler) undoubtedly has been proved to be an obligate parasite on Palmyra Palm in India, and on Coco-nut Palm in both the West Indies and India. Under suitable conditions for growth and spread we might be justified in assuming that this fungus would be the deciding factor in any epidemic, though this must be proved by inoculations on mature trees before final conclusions can be stated.

We must express our deep indebtedness to Dr. Fletcher, Bacteriologist to the Medical Research Institute, for his kindness in examining the bacterial cultures used by us in our inoculation experiments.

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EXPLANATION OF PLATES I-VII.

Illustrating Mr. Sharples' and Mr. Lambourne's paper on Observations in Malaya on Bud-rot of Coco-nuts.

PLATE I.

Fig. 1. Section of fibrous tissue below bud, showing extension of rot into tissues below the bud.
Figs. 2 and 3. Typical appearance of heart-tissues in advanced cases of Bud-rot.

PLATE II.

Inoculated tree in Group G (*Bacillus flavo-coriaceus*); central leaves leaning before falling.

PLATE III.

Inoculated tree in Group C (*Sarcinomyces*); central leaves fallen.

PLATE IV.

Control tree in *Phytophthora* controls; central leaves fallen.

PLATE V.

Inoculated tree in Group A (*Phytophthora faheri*); central leaves fallen.

PLATE VI.

Inoculated tree in Group A; central leaves about to fall.

PLATE VII.

Fig. 1. Showing typical appearance of tree recovering after losing central leaves. The outer leaves have been cut away to show the early stage of the new shoot pushed out laterally.

Fig. 2. Shows appearance of aborted central leaves after recovering.

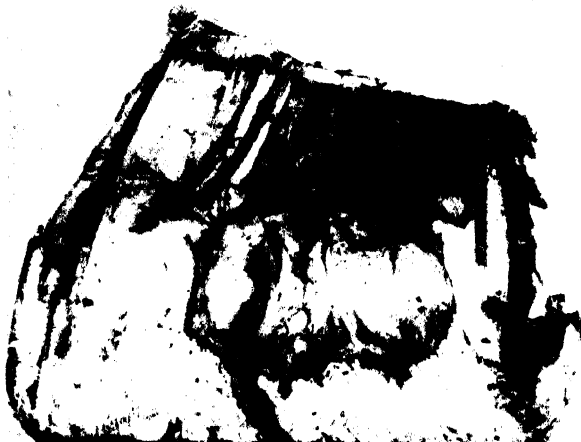
Fig. 3. Successful Bud-rot inoculation with *Phytophthora faheri* (Group A). Only external leaves left standing.

Fig. 4. Central tissues of above tree, showing diseased condition.

1.



2.



3.



SHARPLES & LAMBOURNE — BUD ROT.

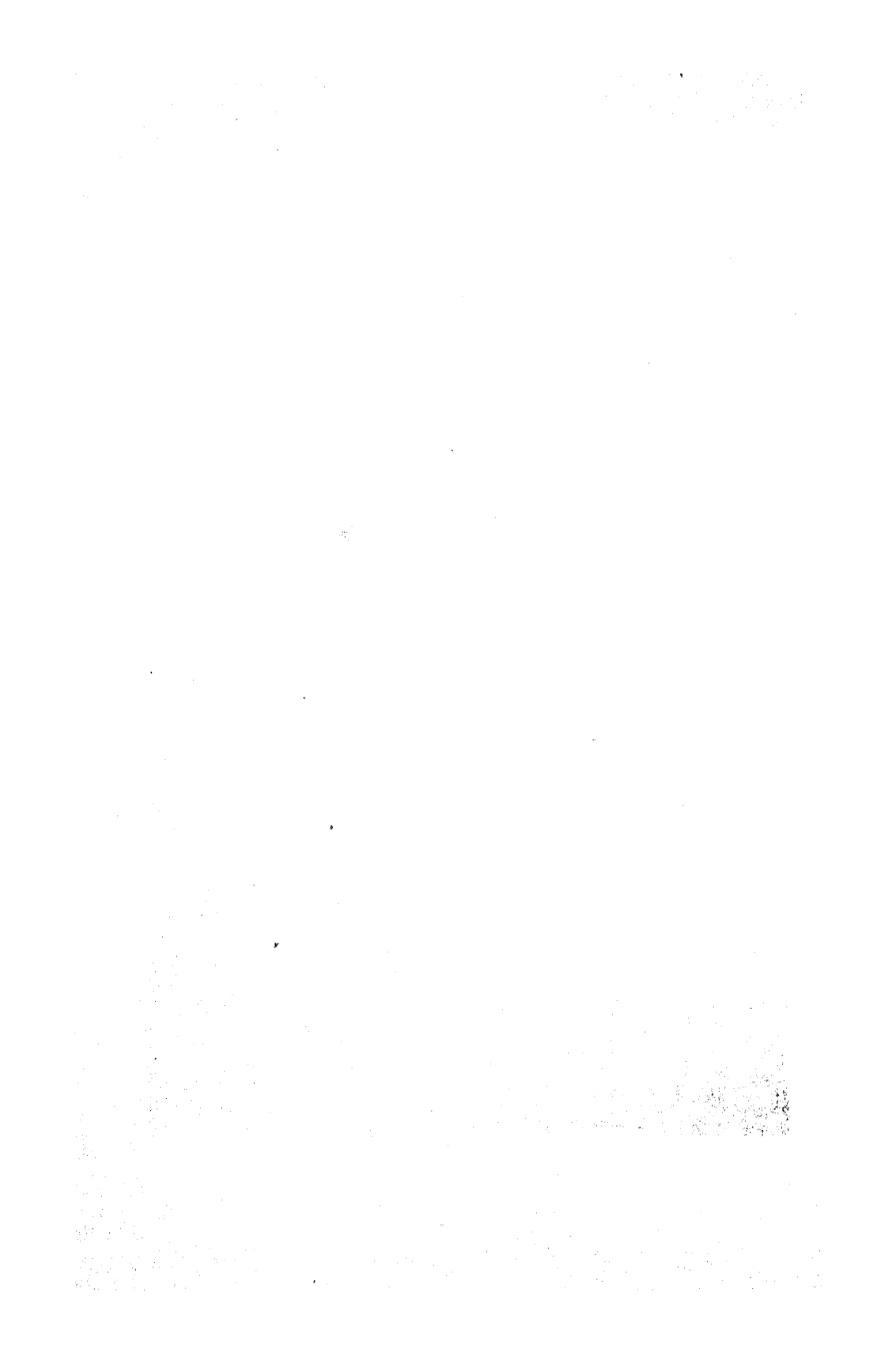




SHARPLES & LAMBOURNE—BUD ROT.



SHARPLES & LAMBOURNE — BUD ROT.





SHARPLES & LAMBOURNE — BUD ROT.



SHARPLES & LAMBOURNE — BUD ROT.



SHARPLES & LAMBOURNE — BUD ROT.



1.



2.



3.



4.

SHARPLES & LAMBOURNE - BUD ROT.

On the Fossil Genus *Sporocarpon*.

BY

R. C. McLEAN.

With Plates VIII-X.

SOME years ago the present writer gave a short description (1) of a group of fossil structures, occurring in the coal-balls of this and other countries, of which the true nature has long been problematical.¹ Minute and complex as these bodies are, they have often been misunderstood, and they have in consequence undergone many vicissitudes of classification since they were first described by Williamson (2). Although they are preserved in a typically vegetable manner, and constantly associated with abundant plant remains, it was thought proper, when the previous description above referred to was published, to establish the several genera as a group of extinct Protozoa. At the same time it was maintained that they should be included in the class Rhizopoda and that their affinities amongst living organisms were most nearly with the Radiolaria.

Notwithstanding this allegation of their animal nature they are historically the property of botanists, since only botanists have written or interested themselves about them, and they are, moreover, constantly passing under the notice of fossil botanists, so that perhaps one may be permitted to introduce an account of them into a strictly botanical journal, as being, so to speak, in our own province.

The question of affinities was fully discussed in the previous article, and it is needless to cover the same ground again, except to point out that no other explanation has offered anything like so satisfactory an exegesis of their structure, and that fresh observations have only tended to confirm the opinion previously advocated, while rendering any of the rival hypotheses practically untenable.

As fossil Rhizopoda (= Sarcodina) these organisms are remarkably isolated, for the only other orders in the class which have been found fossil are, of course, the Radiolaria themselves, and (to a much smaller extent) the Foraminifera; while as an *extinct* group of Protozoa they are unique.

¹ The present descriptions are intended to be *supplementary* to those details which were originally given.

Neither in the fossil Radiolaria nor in the Foraminifera is there any notable change of character between their earliest known forms and those of the present day. In the former case this covers nearly the whole period of the geological record, so that we may conclude that the Protozoa as a class are not only conservative of morphological character, but so fundamentally adaptable that the probability of any large group suffering complete extinction is correspondingly reduced. It is suggested that in these fossils we are dealing with a highly specialized offshoot from a stock normally oceanic in habitat, which had become adapted to life in the vast swampy lagoons of the Coal Measures period, in water, that is to say, probably brackish, and certainly highly charged with organic matter. Under these conditions they underwent a change of nature, the originally mineralized skeleton becoming a chitinated structure, with a consequent degradation of rigidity, while the quiet waters appear to have favoured a higher elaboration of the extra-thecal cytoplasm than is to be found in related pelagic forms. Life in a less highly oxygenated medium would indeed render it obligatory.

If these suggestions as to their origin from known forms of Protozoa be accepted as probable, it makes it easy to understand how liable they were to be extinguished when secular changes of level finally abolished the topographical conditions which had favoured them, and upon which they depended, as a parasite does upon its host, in a balance of adaptation.

The former account, which has been already referred to, included the four genera which were provisionally united into the family Traquairidae; namely *Traquairia*, *Sporocarpion*, *Oidospira*, and *Zygosporites*, while a fifth genus, *Calcisphaera*, morphologically similar, but found in limestones and preserved in calcite, was briefly dealt with, as being probably confamilial with the other forms.

The first of these genera, *Traquairia*, has already been the subject of a memoir by Mrs. D. H. Scott (3), who is collecting further information with a view to more comprehensive treatment on a future occasion. In regard to *Zygosporites* nothing can, so far, be usefully added to what has already been written about it, while *Calcisphaera* calls for a much wider range of investigation, not only in the Carboniferous Limestones, but also in rocks of similar character from other Palaeozoic formations,¹ before a satisfactory presentation of its nature can be made. From the casual notes of its occurrence made by various geologists it appears to be widely distributed both in space and time, and it may have played a large part as a rock-former in the older calcareous strata. While not clearly differentiated in many cases from fossil Radiolaria, or what are accepted as such by geologists, yet its calcareous preservation and some details of its organization appear to give it a claim to separate consideration as an organism

¹ Possibly in Mesozoic formations also, e. g. the grey shales of the Keuper marl.

allied in both directions to Traquairidae and Radiolaria, and probably affording links of connexion between the two. It may be regarded as a parallel development to the Traquairidae, but with a mineralized skeleton. There remain then only the genera *Sporocarpion* and *Oidospora*, which it is here proposed to treat as one, giving a more detailed account of the species, with more complete illustration than was possible in the preliminary treatment, and sketching the probable lines of affinity between them and living Rhizopoda.

SPOROCARPION. Williamson.

1878. Williamson, On the Organization of the Fossil Plants of the Coal Measures. Part IX. 'Phil. Trans.', pp. 169–346.

The delimitation of the genus cannot be regarded as satisfactory, even for a fossil, inasmuch as it is scarcely possible to frame a definition which will reduce the divergent structures to a common denominator. Strictly speaking, almost all the six or more 'species' should be segregated as distinct generic types, and were it not for the fact that each genus so constituted would be practically monotypic this should be done now. Should it become possible in the future to distinguish more than one type within the limits of what is now called one species, as has been done in *Traquairia*, then the larger species thus subdivided will inevitably have to receive new generic titles. For the present, however, there is much to be said in favour of retaining the old name *Sporocarpion* to cover all the forms, since all are obviously closely connected, and, in the absence of fuller knowledge, too fine a subdivision would unquestionably be valueless. It should be clearly laid down at the start that *Sporocarpion* is no true genus, but a plexus of somewhat heterogeneous ingredients, imperfectly separable from one another and only referable in one or two particulars to a common type. The error of nomenclature is even greater in this case than in fossil plants in general, since so much depends, in lower organisms, on cytological details of the plasma, here no longer available as criteria.

The genus *Oidospora*, founded by Williamson at the same time as *Sporocarpion*, differs from the latter only in its smaller size. Indeed, it is not improbably merely a juvenile stage of *S. compactum*, though this cannot be regarded as established. It is monotypic, and it does not seem possible to frame any definition of the larger genus which will exclude the smaller, save only on the point of size, which is, on the other hand, so variable within the genus *Sporocarpion*, as at present constituted, as to make it unwise to lay stress upon it as a generic distinction. Considering this close approximation I propose to suppress its generic isolation and to treat it here as a species of the *Sporocarpion* group under the name of *S. Oidospora*.

To define the limits of *Sporocarpion* brings us against considerable difficulties. In my first account of the Traquairidae I gave a short

definition intended to cover all the known genera, but to lay down a stricter definition of one alone is less easy, if it is to be carried into any particularity. Nor is it a matter of much importance, if we bear in mind the reservations made above regarding the status of the genus.

Confining ourselves, then, to the widest generalities, the following is the taxonomic basis of the forms selected for description here. The description is, of course, subsidiary to that previously given for the group.

Sporocarpon.

Spherical or spheroidal organisms of minute but very variable size (0.03–0.35 mm.); without attachments, and surrounded by a stereomatic envelope composed of vesicles which may be radially or tangentially developed. These vesicles contained protoplasm and possessed the power of extension and development during the life of the organism. Reproduction as in the group as a whole.

Without proposing to split the genus up into new genera it is a matter of convenience to subdivide it into sections, the better to exhibit the interrelationships of the species.

These are as follows :

- | | |
|-------------------------------|-----------------------------|
| <i>Sporocarpon</i> | § <i>Eu-sporocarpon</i> (i) |
| | <i>S. compactum</i> . |
| | <i>S. tubulatum</i> . |
| | <i>S. Oidospora</i> . |
| §§ <i>Fredaia</i> | (ii) |
| | <i>S. elegans</i> . |
| §§§ <i>Diadematis</i> | (iii) |
| | <i>S. cellulosum</i> . |
| §§§§ <i>Sirion</i> | (iv) |
| | <i>S. asteroides</i> . |
| §§§§§ <i>Perichoderma</i> (v) | |
| | <i>S. pachyderma</i> . |

In the classification originally proposed, only one subgenus, *Perichoderma*, was recognized, including the three latter species as given above, but they are obviously so divergent that nothing much was thus to be gained in clarity over the integral genus *Sporocarpon*. Consequently it has seemed better now to subdivide them further, while retaining the old generic title for all.

We will proceed to their description in order :

§ *Eu-sporocarpon*.

- (i) *S. compactum*. Williamson, 'Phil. Trans.', pp. 169–349, 1878.

I look upon this as the central and typical species, as it is the most generalized type, and the others can be satisfactorily regarded as declensions or modifications from this as a primitive form.

The external envelope is formed, in the young state, of cylindrical cells, closely contiguous in the tangential direction and arranged with their long axes radially. There is only one layer, and the outer extremities of all are more or less abruptly acuminate. Although they are almost truly circular in cross-section there are no interspaces between the cells, for the spaces between the lines of actual contact are filled in by extra thickening of the walls, and the cells are slightly broader at the distal ends. In most specimens, and in those the best preserved, the outer pointed extremities of these cells or vesicles bear small dark papillae which may be seen to have finely tufted points, if a high magnification be used. By comparison with *Traquairia*, in which all the radial spines bear fibroid processes, which are clearly hollow, and serve to connect the interior lumen of the spine with the outside, it seems legitimate to conclude that these minute papillae are of similar nature.

The centripetal ends—the bases—of the cells abut upon the wall of the sphere, which is smooth and often fairly thick. The average length of the cells of the envelope is only about one quarter the diameter of the spherical interior space. The question of proximal openings into the interior cavity has not been at all easy to decide in this species. In some of the others, and in *Traquairia*, there can be no doubt that such do exist, a fact which strengthens greatly the unicellular view of the organism. Extremely thin, perfectly radial sections are scarcely possible, and it is generally in a somewhat thick tangential section, giving a hemispherical view, that the structure of the sphere wall is best seen. Those that have been available to me have shown no sign of any large openings, but in several specimens the sphere wall and the radial walls of each cell for a short distance up from the base can be seen to be covered with a very fine punctate marking, like the 'dots' on diatom frustules and just about as minute. Quite possibly this may have been a porose sieve-area which permitted junction of the protoplasm between the cells, and with the interior cavity.

That the cells of the envelope did contain living matter it is impossible to doubt, for they show a marked power of radial elongation. In older specimens many of the cells will be found lengthened into spines, three or four times as long as the original cells. These spines are rather obtuse, never acuminate as in the embryonic condition, so that it is evident that in growing the pointed apical portion of the cell must have shared in the extension of surface, and not have been merely pushed outwards from within by the intercalary elongation of the thinner radial walls. The whole mature spine, above the level of the surrounding cells, tapers gradually, and is covered with the minute nipples spoken of before, whose porose nature can sometimes be clearly seen. Some at least of these must have been added during the secondary growth of the cell. No instance of all the cells of an individual becoming elongated has come to my notice. Usually

only three or four are present in any given section, often grouped closely together, which is a clear distinguishing character between this species and *S. elegans*, where all the peripheral cells develop, though not all at the same time.

This elongation demonstrates clearly that protoplasm lived in the cells of the envelope; and, secondly, that the walls, even the thickened external walls, were not merely skeletal as in the Radiolaria, but were formed of organic material capable of extension. It is probable that the secondary growth of the cells was conditioned by the demand for an increased number of pores of communication in the adult reproductive organism, since it is through these atria that absorption would be chiefly carried on.

The whole organism bears a certain resemblance to *Volvox*, though with obvious differences. The formation of reproductive cells in the interior cavity, unless the protoplasm migrated there centripetally, hardly seems compatible with colonial nature. Most species show clearly that the various spaces of the organism were in open communication, and the localization of reproduction in the large interior cavity suggests strongly that this was a living part of the organism, not merely a mucilaginous medulla. No segmentation of the contents has been noticed except at sporulation, so that all the evidence points to their being truly unicellular, or at least coenocytic organisms. The method of reproduction in all the species is essentially similar, so that one description will serve for all.

Within the outer wall of the sphere comes a delicate 'capsule wall' normally in close contact with the sphere wall, but often shrunk away from it. This may bear fine markings, and is sometimes apparently formed of two separable thicknesses. The capsule contains a closely packed mass of thin-walled vesicles (the so-called *sporoids* of my first account), frequently embedded in frothy *periplasm*. Commonly these sporoids are empty, but one frequently finds specimens in which each one, more or less, holds a small dark-walled *spore* with a sculptured coat. These last may be fungal, but their mode of occurrence leans me to the view that they are the real reproductive cells; the sporoids being of the nature of mother-cells. There is no evidence to show how they were dispersed.¹ Diminutive or otherwise juvenile individuals have no sporoids in their capsules, spore formation only taking place at maturity.

Very little can be said about the subsequent history of these spores. Truly embryonic stages in the development of the spheres have not been recognized with certainty, and I am inclined to think that they were not, until approaching maturity, of a suitable consistency for fossilization. It is possible that *S. Oidospora* may be a young stage of

¹ Since the above was written I have received a very interesting drawing from Mrs. Scott (Fig. 9), in which a specimen of *S. asteroides* is shown (Scott Collection, 1788) apparently extruding the contents of its capsule through a dehiscence of the envelope, not by means of any definitely organized aperture.

S. compactum, and questionable juveniles of *S. tubulatum* and *S. pachyderma* have been found, but there is nothing to add on this point to what was said at the beginning of my first account, i. e. that the spores are apparently asexual autospores, developing directly into mature individuals by increase of size. Minute vesicles are apparently responsible for the markings on their surface, and these are thought to develop into the envelope 'cells' of the mature organism.

Average dimensions :

Total diameter, 320 μ .	Diam. of cells: Base, 16.5 μ .
Internal sphere, 220 μ .	Near top, 22 μ .
Length of cells, 50–60 μ .	Diam. of sporoids, 25–45 μ .

(ii) *S. tubulatum*. Williamson, 'Phil. Trans.', pp. 169–349, 1878.

This species is anomalous in being oval, though the difference in length of the two axes is not very considerable. All the same it affords a parallel to *Zygosporites oblongus*, although not so markedly elongated, and thus helps to illustrate the homogeneity of the group. Taking an average of the few specimens available, the greater diameter is about one-eighth more than the lesser.

The structure of the envelope in *S. tubulatum* is superficially similar to that in the preceding species, so that it is not surprising that it was originally named as a variety of *S. compactum*.¹ In the young condition there are contiguous tubular vesicles all over the exterior surface, their long axes disposed radially, as in the first species, though here the vesicles are nearly five times as long as broad. They are slightly broader distally, and their outer ends, instead of being sharply pointed, are smoothly rounded off, without any papillae or openings of any kind.

At the base of each such cell is a well-marked pore communicating with the interior of the sphere. Indeed in this species this form of opening is better shown than in any other. There can be no doubt that free interplay of living material existed between the interior cavity and the peripheral cells during life.

It is remarkable that both in this and the former species the cells of the envelope are very constant in size and regular in their adjustment each to the other. Just as in *S. compactum*, the vesicles of the envelope matured into long spines; but here we have definite evidence that they all elongated as in *S. elegans*, although probably in succession, not simultaneously.

Maturation of the spines was accompanied by swelling out into a globose bulb, at about the summit level of the young vesicles, in such a way as to thrust aside the neighbouring ones and clear a space round the proximal portion of the new spine. The apical half tapered regularly to a sharp, solid point, without any sign of perforations on its wall, the whole mature spine being more than twice as long as its parent vesicle

¹ Williamson, in his slide catalogue, not published.

and equal to more than half the diameter of the whole organism. A slight constriction develops just above the basal pore. In the oldest stages all the apical moiety of the spines may get knocked off, leaving only the disorganized basal portions. This can hardly have taken place during life, for the protoplasm would have been thus exposed through the broken ends. The breaking off of the spines in this way, found similarly in *S. elegans*, is evidence of fragility, which we might well predict from the general delicacy of the walls throughout development, but which is in striking contrast to the great flexibility that is evident in the spines of *Traquairia*.

A somewhat rare species. I have not seen more than half a dozen specimens, and none of these contained any reproductive cells or capsule. In size it is somewhat smaller than *S. compactum*, even in its greater diameter, though the spines are much longer, both relatively and absolutely.

The following are the principal elements:

Diameter over all :	Length of vesicles, (<i>circa</i>) 67 μ .
Greatest, 286 μ .	Breadth, 16 μ .
Least, 276 μ .	Length of mature spine, 155 μ .
Diameter of interior :	Breadth at swelling, (<i>circa</i>) 25 μ .
Greatest, 149 μ .	Proximal pore, 2.5-4 μ .
Least, 133 μ .	

(iii) *S. Oidospora*: mihi. *Oidospora anomala*. Williamson, 'Phil. Trans.', pp. 169-357, 1878.

This species is anomalously small, but otherwise seems to fall suitably into line with the two preceding organisms, in the present class. It consists of a spherical central portion, enclosed within an envelope of somewhat rounded vesicles, as closely set as their shape permits, and flattened at their bases, where they are attached to the wall of the sphere. No perforations have been observed, either to the exterior or to the interior, and the walls are all perfectly thin and smooth. The vesicles of the envelope are about equal to the diameter of the sphere in length. Although somewhat like *S. compactum* no intermediates between the two are known. The present species is probably therefore a substantive one.

Only two preparations have come under my notice, so that it is probably the most infrequent of the known forms. One of these is in the Williamson Collection at the British Museum (section numbered in catalogue 1552), containing large numbers, from among which Fig. 102 in Williamson's ninth memoir was taken. The other slide, containing only a pair, is in the University College London, Collection. Like most of the others, this species appears to have been gregarious, judging from the large numbers on one slide, but there is no definite sign of union into colonies. On slide W. 1552 there are a few individuals with markedly enlarged and elongated vesicles, suggesting, though not conclusively, elongation into spinous processes. No interior capsule or spores have been found.

*Dimensions:*Total diameter, 55 μ .Length of cells, 14 μ .Diameter of sphere, 28 μ .Breadth of cells, 10 μ .§§ *Fredaia*.*S. elegans*. Williamson, 'Phil. Trans.', pp. 169–348, 1878.

This was taken in my last paper as the type species of the genus, but I now refer *S. compactum* to that position, as the least specialized type. The present organism has an envelope formed of tapered spines, with a median and a proximal swelling, between which—that is, in the lower half—the shaft is constricted to a slender stalk. The median and basal swellings are contiguous all over the sphere, so that there is formed a 'colonnade' of the slender portions of the shafts and the continuous space which lies between them. As in *S. tubulatum* the spines must have been somewhat brittle, for only a few survive at full length even in the best-preserved specimens. They were broken off all about the same level, just above the median swelling, and it is easy to see, by the broken stumps which form the greater part of the envelope in most specimens, that originally each cell of the envelope formed a spine. Where the 'cells' of the colonnade touch one another their walls are thickened, so that where the spines break off, just above this level, there is a thickened honeycomb-like framework left behind at the outer surface of the colonnade layer. (See Pl. VIII, Figs. 1 and 3.) Occasionally spines may be observed flexed, but not often. The walls of the spines are perfectly smooth and free from perforations, but the tips are often bifid or trifid, with widely divergent segments, a character which has its counterpart in *Traquairia*. The lumen connects with the interior of the sphere by a single pore.

Williamson believed the spaces of the 'colonnade' to be filled by thin-walled cells, relying upon the appearances shown in tangential sections. I myself, finding that the walls of these 'cells', cut in tangential sections of the envelope, were double throughout, came to the conclusion that they were really the remains of a system of tangential tubuli, homologous with those of *Traquairia*, connecting up the proximal ends of the 'colonnade' units. The spaces of the 'colonnade' are not, I believe, occupied at all, but the appearance of traversing walls or tubuli may possibly be due only to the overlapping of the margins of the basal swellings, which can easily be observed in transverse section, and which may lead to this appearance of tangential connexions when seen from above.

The species is a fairly common one, and is often singularly plentiful when met with. Good specimens well deserve the specific name. Curiously enough, no specimens have been found which throw any light on the development of *S. elegans*, although we have good evidence of the history in both *S. compactum* and *S. tubulatum*. There is little doubt that the

young stage must have been very like the young stage of the former species, and may have been confounded with it.

The interior capsule and sporoids have been found very well preserved, with abundant periplasm. In the spines of this species, as well as others, I have sometimes seen very small spheres, not unlike the spores. They can hardly be true spores in such a position, and I believe they may be commensal protophytes, analogous to the testiculate flagellata sometimes found in modern Radiolaria.

Dimensions:

Total diameter, 480 μ .	Width at swelling, 25-35 μ .
Diam. of sphere, 360 μ .	Width at base, 25-35 μ .
Length of spines (base to tip), 180-240 μ .	Width of constricted part, 8-9 μ .
	Height of colonnade, 50-65 μ .

§§§ *Diadematis.*

S. cellulosum. Williamson, 'Phil. Trans.', pp. 169-347, 1878.

In this subgenus the true structure of the envelope is in some doubt. So far as can be judged there appear to have been divaricate spines in groups round the periphery, each group having something of a tiara-like appearance in side view. I have never seen any specimen in which these spines were fully developed, and it is open to question whether any elongation took place further than what has been observed. In this condition they form stout, comparatively short tubes, with dark and rather thick walls, blunt and open distally. Each group consists of longer tubes in the middle, the remainder declining in length outwards, in both directions, giving the impression of a kind of sorus with basipetal development.

That development did actually run in this order seems evidenced by the way in which the neighbouring elements of the envelope are deflected from the truly radial position, pushed away as it were by the elongation of the elements of the sorus. Between these widely separated groups of spinous processes the envelope is composed of similar, but quite short tubes, evenly distributed. In tangential section all appear to be hexagonal and close fitting, but in radial view the appearance is that of alternating cells and vacant spaces.

Running tangentially between all these tubular processes are several series of fine dark lines. These may be either very fine tubules themselves, or thickening bands upon the radial cell-walls, or, more probably, pairs of them may be the edges of comparatively stout tubes in longitudinal section, or they may be the walls of true cell elements, filling in the interspaces between the radial tubes. As I said before, the specimens available have not been sufficient to elucidate the point, though I incline to the second view. If they are really cells, there would be afforded a welcome link with the structure of the following species, which otherwise is extremely isolated.

Proximal pores communicating with the interior have been seen at the base of the radial tubuli. Central capsule and reproductive cells normal.

No certain developmental phases have been observed, but a spherical body with an envelope of a single layer of small square cells with dark walls, once found, suggests a possible juvenile form.

Dimensions:

Total diameter, 400–420 μ .

Diam. of sphere, 350 μ .

Length of best-developed processes, 65–70 μ .

Width of such processes, 20–23 μ .

Length of short processes, 15–20 μ .

§§§§ *Sirion*.

S. asteroides. Williamson, Phil. 'Trans.', pp. 171–510, 1880.

A strikingly peculiar species, in which the envelope consists of a firm parenchymatous tissue, or what appears to be such, though it may actually be paraplectenchyma, the difference being obscure even in isolated sections of living plants, and almost indecipherable in a fossil. The great regularity in size and arrangement of the cells does, however, look very like true parenchyma. The envelope is produced into large lobes, nearly as long as the organism is wide, five or six of which may appear in any section. Hence the specific name.

The walls of most of the cells are quite thin, except at the outer surface and in particular at the tips of the rays, where there is marked induration and darkening. Faint markings are sometimes discernible on the walls, but no cell-contents have been seen. The inner layers of the envelope are sometimes markedly meristematic in appearance, and this, in conjunction with the thickened walls and absence of divisions in the outer layers, points clearly to centrifugal growth of the envelope. The outer surface is very seldom well preserved; in fact, the only perfect outer surface I have seen, and that one irregular, was in a young specimen with very small lobes, which suggests that the outer surface was subjected to erosion during later life and was worn away and renewed internally like so much periderm.

The average depth of the envelope is 5–6 cells, but a lobe when mature may be more than a dozen layers in depth, while in the bays between lobes there may be only one layer. No perforations of any sort are visible, and it is rather difficult, with this in mind, to envisage any but a holophytic method of nutrition.

The capsule wall is peculiar, for it can be clearly seen under high magnifications (500 diam. *et supra*) to be patterned with very minute and closely set dots. In transverse section these dots show up as raised papillae on the outer surface of the capsule wall, while especially favourable examples

show a complementary jutting of the inner surface of the sphere wall. The sporoids and periplasm are exactly as in other species.

Williamson described and named as species of *Sporocarpon* (*S. anomalum* and *S. ornatum*)¹ two objects with just such a parenchymatous investment as this, which have since been shown to be the transverse sections of small Pteridospermic seeds.² Indeed they show many features which might have aroused suspicion of their nature. One might easily dismiss the present species as an impostor and thus be rid of a peculiar difficulty, were it not for the very clear evidence of the capsule and its spore contents, which are perfectly in accord with the same structures throughout the group.

Similarly, *S. asteroides* agrees with other species in secondary points; in the matter of size, for example (though this is rather variable, within limits), and in its gregarious habit, so that I cannot but regard it as an established member of the genus, albeit not easy to bring into line with the remainder.

The diameter of the sphere is rather more variable than is usual, although all species exhibit some plasticity in this respect, strengthening the view that the interior cavity is an integral living part of the organism. Growth is straightforward, the original form being without projecting lobes, which develop secondarily, but early, upon a narrow, spherical envelope of cells.

Dimensions:

Width of sphere, 180–330 μ .

Length of lobes, 120–140 μ .

Width of lobes at base, 90–120 μ .

Average thickness of envelope between lobes, 25 μ .

Size of individual cells, 8–16 μ .

§§§§§ *Perichoderma.*

S. pachyderma. Williamson, 'Phil. Trans.', pp. 171–510, 1880.

An interesting and frequent species. The envelope is composed of large interlacing tubules, anastomosing closely with one another in all directions. Neither beginning nor end is traceable for any given tubule: all are fused into a closed system round the stout wall of the sphere. At the external surface certain tubes form extruding apertures which there is reason to believe may have formed the bases of radial processes, usually lost or crushed, but as they stand they merely provide communication between the lumen of the tubular network and the outside. Usually all the ramifications are approximately of equal diameter, but there is a second type distinguishable where certain larger and darker walled tubes form

¹ Phil. Trans., 1880 and 1883.

² Oliver, Ann. Bot., xxiii. 74, 1909. The statement that *S. anomalum* is a mere slip for *S. ornatum* is not correct, for the original specimen, W. 1326, is so labelled by Williamson. Nor are the two altogether identical, though the differences may be due to their respective states of preservation.

a wide meshwork, as viewed in tangential section, the interspaces being filled in with slighter tubules. The larger tubes in this type bear the external orifices at the nodes of their mesh. These two forms are not, however, sufficiently distinguished by this peculiarity alone to warrant their specific segregation from one another. All the tubes are quite thin walled, and there is no appearance of contents, or of finer lateral openings, or appendages such as might be suggested by the analogy of the investment of tangential tubules in *Traquairia*. Openings to the interior exist, and the capsular contents of sporoids and small spores are quite normal, except that the capsule wall may show a very delicate reticulation (slide 1503, Williamson), an appearance also noted in *Traquairia Carruthersii* (W. Coll. 1066, 1074, 1077). The capsule sometimes shows a double wall, but this is met with occasionally in all species and may be the universal condition. On the other hand, the external surface is peculiarly interesting. It often appears to be covered with a dark membrane, double in places, through which the exoscopic apertures thrust themselves, while many specimens have small thin-walled vesicles like bubbles, borne attached organically to the outside of the tubular envelope.

Putting these aside for the moment, let it be noted that the species is not only highly gregarious, but actually colonial, several individuals being massed together in one confluent envelopment of tubes. This marks a distinct departure from the usual Traquairidean type and bespeaks an approach to the Pharetrone sponges. Many cases of confluent individuals show one or more of the attached individuals collapsed, and when this is the case the collapsed one always has a very exiguous envelope of tubes, with a dark and well-marked sphere wall. Now I would suggest that these are the younger members of the colony, as yet unstrengthened by the full development of their envelope, and that they are formed on the outside of older members by the growth of the round vesicles previously spoken of, building up a sort of *Globigerina*. Thus the colony would be regarded as formed by superficial budding, each new sphere forming from an enlargement of one of the peripheral tubes in the older organism; which is in a manner a parallel phenomenon to the secondary enlargement of the cells in § *Eu-sporocarpon*.

One very peculiar specimen (U.C.L. Collection), mentioned in my last paper, shows three confluent individuals, in one of which there is an internal ring of wide oblong cells arranged radially, with very dark contents. These are very puzzling and suggest further uncomprehended possibilities in regard to the group as a whole (Pl. X, Fig. 19).

The lumen of each cell is confluent outwardly with a tube of the envelope, so that they are part and parcel of the investment system and not special reproductive cells of any sort.

Taking into consideration the colonial habit, I would hazard the view

that this is the mother-cell of a colony, and that the ring of radial elements is the precursor in development (from the spore) of the tangentially arranged tube network, and therefore only found in the mother-cell, while absent from the proliferous outer daughter-spheres of the colony. Such a scheme affords a useful hypothesis, and serves to exhibit a possible connexion of this anomalous form with the typical envelope of radial vesicles in § *Eusporocarpon*. It will be desirable to advert to these possible affinities later on.

A very probable spore, with irregular wall, has been found in close proximity to a large nest of individuals (see Pl. IX, Fig. 14).

Dimensions :

Total diameter, 330–390 μ .

Diam. of sphere, 240–270 μ .

Average thickness of integument, 60–90 μ .

Average width of tubules, 8–16 μ .

INTERRELATIONSHIPS.

It would be fallacious to try to unite these few species into anything like a phyletic sequence, but it would not be undesirable to emphasize here the points in which they seem to connect with one another.

Taking *S. compactum* as the standard, we can see that separation of the vesicles from one another and grouping of the scattered spines into 'sori' would produce a type resembling *S. cellulosum*. Again, complete development of the vesicles into spines, as shown in the closely allied *S. tubulatum*, leads us to the *S. elegans* type, where the constriction of the basal moiety of each spine—indicated in one peculiar example of *S. compactum* itself (T. 8, in my own possession) and normal in *S. tubulatum*—has been carried to the extreme. As before stated, *S. asteroides* stands somewhat apart. Its nearest relative would appear to be *Traquairia stellata*, R. Scott, where the spinose arms are divided into cellular compartments, but I think the two are really parallel developments, and not genetically connected. *S. asteroides* might be reached by a proliferation of the juvenile vesicles of *S. compactum* into a pseudo-parenchyma, in place of their normal individual development into spines.

S. pachyderma, in the light of the specimen with an inner radial investment of cells, is best regarded as a derivation from the *S. compactum* form, in which a colonial habit has been established and a general envelope formed by the prolongation and anastomosis of the distal portions of the radial envelope cells into a complex outer stereome of tubules, beyond which their free apices may have projected. Obviously this yields points of contact with the envelope of *Traquairia*, where ramification of the spines is general. But the envelope is formed by the anastomosis of proximal rather than distal ramifications. Postulating a type of *S. compactum* in

which every vesicle was developed into a spine (one of Williamson's specimens approaches this condition), we would possess a feasible working hypothesis of a prototype from which not only the remaining species of *Sporocarpion* may be derived, but which might give indications (chiefly through *S. cellulosum*) of the origin of the more specialized genus *Traquairia*.

RELATIONSHIPS TO OTHER PROTOZOA.

The comparison of the genus as a whole with living Protozoa is not easy, for there is no resemblance in an aggregate of particulars such as to point indubitably to one group with which detailed morphological homologies might be shown. There are indications of affinity to both Foraminifera¹ and to Radiolaria, but especially the latter. Not that these present real homologies with the Traquairidae. At most it can be said there are suggestive analogies. Chiefly interesting, perhaps, is the central capsule, in the Radiolaria the seat of the nucleus and of sporulation. A fundamental divergence is the absence in these fossils of any system of perforations of the capsule wall, unless the fine punctations occasionally observed are to be so interpreted, in which case their affinities would obviously be with the primitive group of Peripylaria, possessing an evenly porose capsule. Association with the Peripylaria raises naturally the question as to whether we are not dealing in a *Sporocarpion* sphere with a colony of individuals, after the fashion of *Collozoumi inerme*, each sporoid being then regarded as the homologue of a complete central capsule. I have already indicated that the balance of evidence is against this opinion, but it cannot altogether be discounted. The spores, again, present difficulties, for the spores of *Sporocarpion* are furnished with a palpable wall, enclosing protoplasmic relics, while those of modern Radiolaria are quite unsubstantial and indeed self-motile. Here a comparison with Foraminifera may help us out, for among them the reproductive amoebulae surround themselves with a minute one-chambered cell as the starting-point of a new individual. How the spores were exerted from the interior chamber of the organism there is no evidence to show, unless they may be imagined to escape by the dissolution of the envelope.² This envelope appears to be held together chiefly by its attachment to the sphere wall, but the sphere wall may not have been so substantial as it appears, for it may be looked upon as the correlative of the dark fatty assimilative layer of protoplasm closely surrounding the capsule in the Radiolaria, and thus perhaps much more readily disintegrated than a solid non-plastic membrane would be.

¹ The relationship to Foraminifera has not been considered here so fully as that to Radiolaria, but it may be pointed out that the genus *Polytrema* has several features suggesting affinity with the present group.

² But *vide supra*, p. 76, and Fig. 9.

The Radiolaria form both isospores and heterospores, the latter being sexual gametes, and some species even show a complete dimorphism according to the type of spore formed. Among the Traquairidae isospores are predominant, but specimens frequently show spores of different size which may be true heterospores, or may be mere irrelevancies due to fungal intrusion during decay. In some cases it is very difficult to accept this latter explanation (*vide Traquairia* in W. 1063).

One further point of interest presents itself. There is only one class of Radiolaria in which no 'zooxanthellae' (endobiotic Chrysomonad flagellates) are present, namely, the Phaeodaria. Among the Phaeodaria we find close analogies in form and composition with the skeleton of the Traquairidae, but each individual invariably contains a large dark excretory aggregation. This is quite absent in our fossils, unless the dark mass in the capsule of *S. elegans*, Pl. VIII, Fig. 3, be so interpreted, so that one can scarcely associate the two groups very closely, but at the same time the absence of this aggregation of excretory granules raises the interesting possibility that endophytic algae may have played a part in the life-history of these fossil organisms as in the true Radiolaria. The development of elaborate systems of external apertures leaves little doubt that their main method of nutrition was, as in the Radiolaria, holozoic, but variation in this respect is probably evidenced by the structure of *S. asteroideus*. The skeletal affinities were treated pretty fully in my first paper. I still maintain that a chitinous substance seems the most likely material. Evidently it was something pretty hard, since the preservation is meticulously accurate in the smallest details, and it was in most cases flexible.¹ Among the Phaeodarian Radiolaria the skeleton often suggests the Traquairian form, and it consists of an organic silicate, which is at least nearer in nature to the fossils than is the strontium sulphate skeleton of the Acantharia. At the same time the internal arrangements in the present group are much more nearly those of the Peripylaria. Therefore I would suggest that the nearest living analogues of the Traquairidae are to be found in the group Orosphaeridae, removed from Phaeodaria to Peripylaria by Häcker (4), which shows a union of the characters of the two groups parallel to that observed in the Traquairidae.²

Since, as Cavers (5) has pointed out, the Radiolaria may be derived from ancestors like the Gymnodiniaceae, it is not improbable that the Tra-

¹ The Foraminifera possess a chitinous test between the protoplasm and the calcareous test. This may develop excessively, not as a consequence of starvation or wandering into brackish water, as formerly suggested, but as a variation in any species under any conditions (Heron-Allen, Rep. Brit. Ass., Bourne-mouth, 1919). Wholly chitinous forms may appear as monstrosities.

² The Coccolithophoridae (see Murray and Blackman, Nature, lxx. 510, 1897) must also be considered as possible descendants, on the evidence of their armour characters, though their cytology is not fully known. Indeed, I would be nothing loath to postulate a common plexus of origin for the Coccolithophoridae, Traquairidae, Radiolaria, Diatomaceae (as represented by the early liassic genus *Ptychodictya*), Prorocentraceae, and Peredineae.

quairidae may have sprung from some similar group of Peredinian affinities, with spores possessing a hard cell-wall, in place of the lower, flagellate type represented by the spores of Radiolaria. It has been maintained all along that their homoeomorphy with the latter group is rather the result of parallel descent than of homology.

Sporocarpion pachyderma has particular affinities of its own with the Keratosa group among the sponges, and represents an advance in their direction, which brings it into relation to a parallel living group of Protozoa, the little-known Xenophyophoridae (6), which also, though widely asunder, have developments tending similarly to a sponge-like facies.

I have previously suggested that the present group represents a line springing from common ancestors with the Radiolaria, probably not very far back from the present level of that class, the modifications being traceable to life in shallow, highly organic waters with a low saline content. Such habitats have not often been extensive in geological history, but as the Carboniferous Coal Measures are the only period of the sort sufficiently closely investigated, it is impossible to deny the existence of similar organisms at other epochs similarly constituted. Not improbably Mesozoic and Cainozoic coal deposits may yield allied or identical forms when they have been more fully examined. At present, however, so far as we can tell, they are exclusively Palaeozoic and limited to the Coal Measures, though on those horizons they attained a wide distribution. The mineralized skeleton of the pelagic Radiolaria is by no means an insuperable difficulty in the way of associating the groups, since such skeletons are also found in several isolated groups, for example among the Diatomaceae and the Silicoflagellata, without the occurrence of anything but an organic test in their nearest relatives. Fossil Radiolaria are found extensively in Lower Palaeozoic rocks, even the lowest, notably in the Lower Culm of Devon and Somerset (7) and in the European Culm in general, almost at the base of the Carboniferous succession (8). Use has been made, by geologists, of their presence in these rocks to argue very deep water conditions at the opening of this geological period, and a gradual shoaling until the Carboniferous Limestone is reached, with subsequent elevation and the commencement of the Upper Carboniferous rocks.

There can be little doubt of the affinity of the organisms figured by Hinde and Fox (7) both with Radiolaria and with Traquairidae, though clearly their skeletons have undergone resolution and replacement, so much so indeed that the generic and specific assignments awarded them are more than dubious. The interesting point at the moment is the presence of a class of organisms resembling modern Radiolaria, widely and plentifully distributed in the seas preceding the formation of the Coal Measures. From this organismal reservoir it is, as I conceive, that the Traquairidae were drawn, during the subsequent shallowing of the waters.

The genus *Calcisphaera*, too, may have originated in this period, although it makes its appearance earlier, in the Carboniferous Limestone. Hill and Jukes-Browne (9) have shown how unstable chemically is the Radiolarian skeleton during fossilization, and *Calcisphaera* may likewise have suffered chemical replacement of either a siliceous, strontium sulphate, or organic test by calcite. Apart from its chemical nature there is little to separate it from true Radiolaria, and it affords several morphological links between them and the Traquairidae. Taking into account its earlier appearance and its intermediate distribution, in the moderately deep water limestone deposits, it may well be a connecting group.

SUMMARY.

1. The present article is an attempt to give in full detail the facts, in so far as they have been determined, of the structure of the genus *Sporocarpon*, Williamson, one of the group of Traquairidae, previously established (1).

2. The genus is shown to cover a multiplicity of divergent types, represented only by single species, which it is impossible to regard as generically related in the present-day sense. The old generic name is retained for convenience, but five subgenera are proposed.

3. A revised monographic description of the seven species is given. The organism called by Williamson *Oidospora anomala* is added to the present genus under the name of *Sporocarpon Oidospora*.

4. Taking *S. compactum* as the simplest and least specialized type, it is shown that it is possible to derive the structure of the other species from it by divergent changes, while *Traquairia* may also be derived from a *Sporocarpon* type, probably in the neighbourhood of *S. cellulosum*. This emphasizes the homogeneity of the group.

5. The relationships of structure between *Sporocarpon* and recent Protozoa are examined in detail and the conclusion pointed that the nearest living analogues of Traquairidae are the Peripylarian Radiolaria. At the same time the material of the skeleton and the appearance of the spores suggest that, like the Radiolaria, they may ultimately have sprung from flagellate ancestors of a Peridinian type.

6. Evidence is adduced for the widespread existence of Radiolarian organisms at the base of the Carboniferous period in abyssal waters; while in the shallower water limestone deposits of the Lower Carboniferous, the intermediate organisms called *Calcisphaera* are enormously prevalent. The bearing of this upon the phyletic origin of *Sporocarpon*, afterwards typically developed in the non-saline waters of the Coal Measures, is pointed out and suggested as a probable trace of their evolution.

7. These organisms are specially characteristic of sections taken from coal-seam nodules (coal-balls) of the Lower Coal Measures, and in particular

the Burntisland and Halifax Hard beds. It was in the latter that they were noticed for the first time by Messrs. Spencer and Binns.

The sections which I have chiefly consulted are those in the Williamson Collection at the British Museum; the University College, London, Collection; Prof. Seward's Collection at Cambridge, and some in the possession of Dr. D. H. Scott and myself. I wish to express my indebtedness to Prof. F. W. Oliver and Dr. D. H. Scott for the kind loan of their preparations, and to Mrs. Scott for a similar kindness with regard to photographs and drawings.

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EXPLANATION OF PLATES VIII-X.

Illustrating Prof. McLean's paper on *Sporocarpon*.

PLATE VIII.

Fig. 1. *Sporocarpon elegans*. Tangential section, showing the numerous spines arising from the honeycomb-like framework which forms the outer limit of the 'colonnade' layer. Towards the centre the attenuated waists of the colonnade units may be seen in cross-section, and between them their delicate tangential connexions. Tams phot. Lent by Mrs. Scott. This specimen is figured in Williamson, Part X, and the slide is in his collection. $\times 100$.

Fig. 2. *S. elegans*. Median section, showing a shrunken capsule and several spines, the extremities of certain of which (at A) may be seen to branch. Tams phot. Lent by Mrs. Scott. $\times 100$.

Fig. 3. *S. elegans*. Median section, showing the structure of the colonnade of spine base units with exceptional clarity. Capsule and sporoids well preserved. Where the spine bases are in contact

at the region of the intercalary swelling, it will be seen that their walls are somewhat thickened, forming the framework referred to under Fig. 1. Tams phot. Lent by Mrs. Scott. $\times 100$.

Fig. 4. *S. elegans*. A truly tangential section through the colonnade with the units cut at different levels; upper swellings on the periphery of the section, lower (foot) swellings in the centre and 'waists' intermediately. The photograph shows the tangential connexions running between the units, with here and there a double contour, as if they were tubular. (Williamson Collection.) $\times 450$.

Fig. 5. *S. compactum*. Restoration of a single unit (cell) of the envelope, at the beginning of its elongation into the mature spine form. The tuberculae shown near the apex are really minute openings with pores at the tip of each of their very fine ramifications. $\times 2,000$ approx.

Fig. 6. *S. compactum*. Part of the envelope, showing two cells at A in process of elongation. (Author's Collection.) $\times 450$.

Fig. 7. *S. asteroides*. Median section of a specimen with unusually long lobes. Interior capsule faintly shown. (U. C., London, Collection.) $\times 20$.

Fig. 8. *S. asteroides*. Young specimen in median section, showing part of the outer surface of a young lobe at A, with convex outer 'cell-walls'. (U. C., London, Collection.) $\times 80$.

Fig. 9. *S. asteroides*. Two specimens, one containing a capsule with sporoids, the other apparently dehiscing and extruding a mass of spores(?) united by filaments. A unique specimen. Drawing by Mrs. Scott from a specimen in the Scott Collection. $\times 100$.

Fig. 10. *S. tubulatum*. A young specimen in median section, showing the elongate cells of the envelope in their young form before their growth into spines. It will be seen that in this state their apices are obtuse. Compare with Fig. 11. (Author's Collection.) $\times 80$.

Fig. 11. *S. tubulatum*. Restoration of two spines in their mature state, showing their resemblance to those of *S. elegans*. The whole organism is, however, in this species spheroidal. $\times 1,000$ approx.

PLATE IX.

Fig. 12. *S. pachyderma*. Section through a conjoined group of individuals with a common tubular investment. One, which is cut medianly, shows a peculiar colonnade of radial cells, remarkably like that of *S. compactum*, inside the normal tubular envelope. A unique specimen. (U. C., London, Collection.) $\times 75$. See also Fig. 19.

Fig. 13. *S. pachyderma*. Part of the tubular investment shown in tangential section. The branching of the large thin-walled tubes composing the investment may be seen near the centre. (Author's Collection.) $\times 450$.

Fig. 14. *S. pachyderma*. Spore of juvenile individual with very slight investment, found in close association with a large group of the same species. (Author's Collection.) $\times 600$.

Fig. 15. *S. pachyderma*. Median section with double-walled capsule. The envelope shows apparent orifices externally at A and a spine base at B. (Author's Collection.) $\times 450$.

Fig. 16. *S. cellulosum*. Radial section with large 'sorus' of spine bases. A small portion of the envelope is seen cut tangentially (A), showing the polygonal outline of the envelope units.

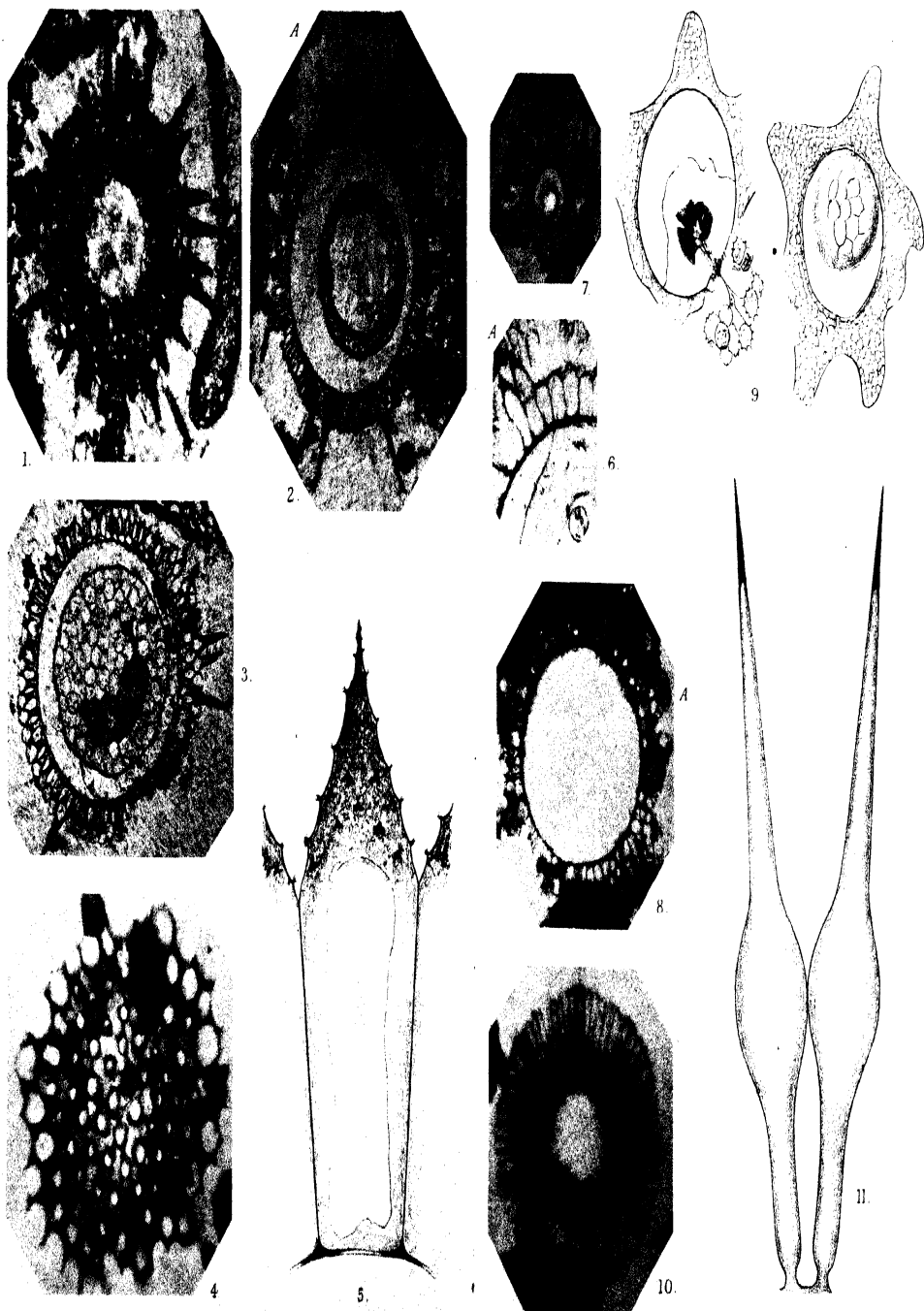
Fig. 17. *S. Oidiospora*. Two individuals, apparently conjoined. The structure of the envelope is very like that of *S. compactum*. (U. C., London, Collection.) $\times 700$ approx.

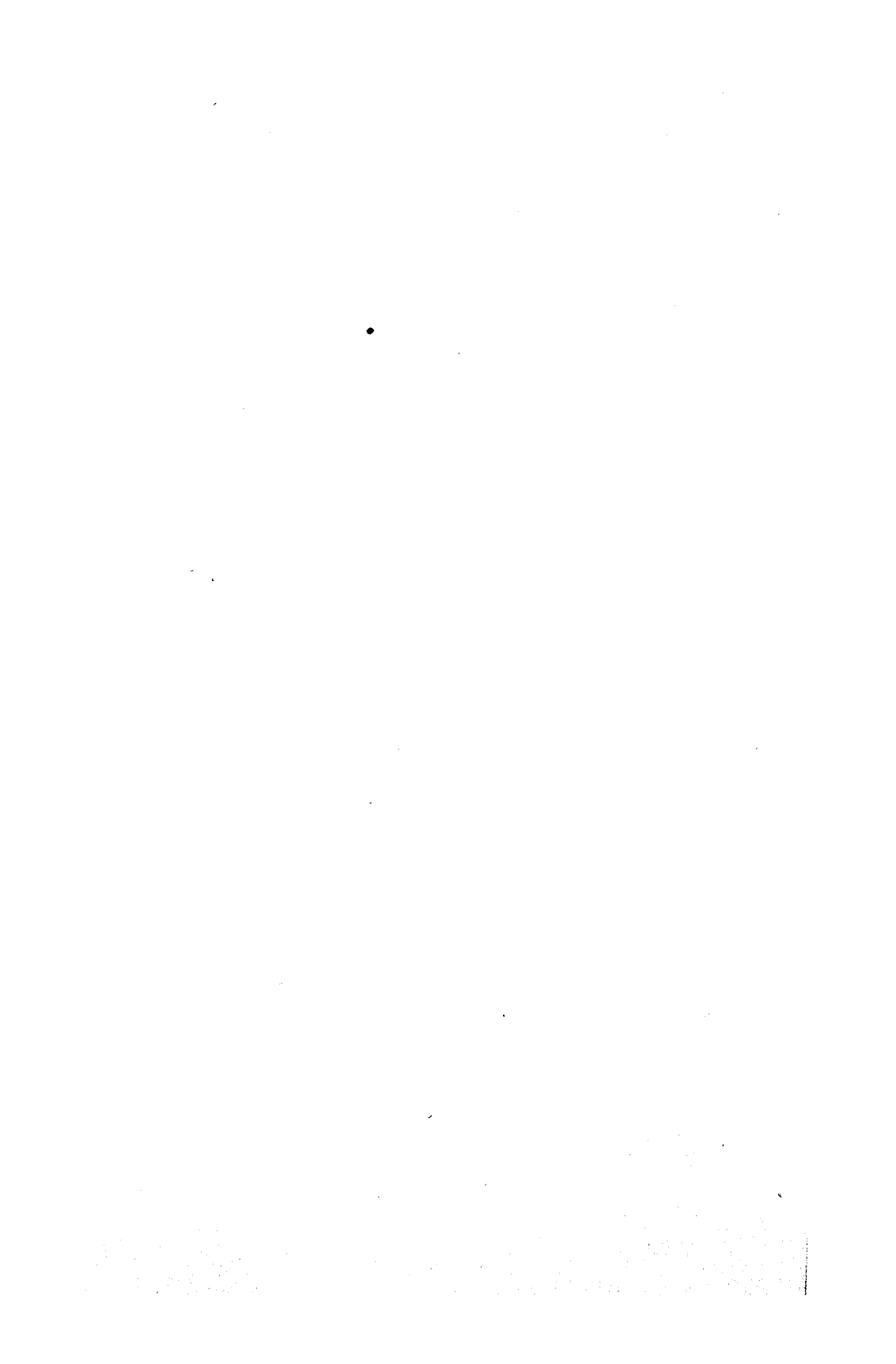
PLATE X.

Fig. 18. *S. compactum*. Median section of a very well preserved specimen with an unshrunk capsule filled with sporoids and granular matter which appears to be residual plasma. U. C., London, Collection.) $\times 700$ approx.

Fig. 19. *S. pachyderma*. Portion of the envelope of the specimen shown in Fig. 12. It will be seen that the radial cells are prolonged at their distal ends into the tubes which form the envelope, the prolongation being in each case flexed in the same (clockwise) direction. $\times 200$ approx.

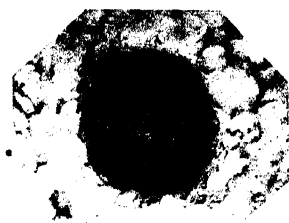
Fig. 20. *Traquairia ramex*. The type specimen of this species, which is here reproduced for comparison with the present genus. (U. C., London, Collection.) $\times 200$ approx.



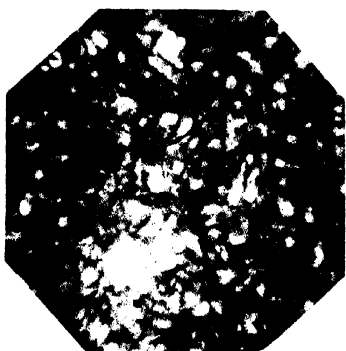




12.



14.



13.

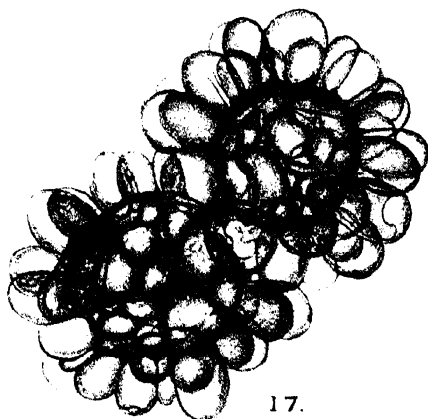


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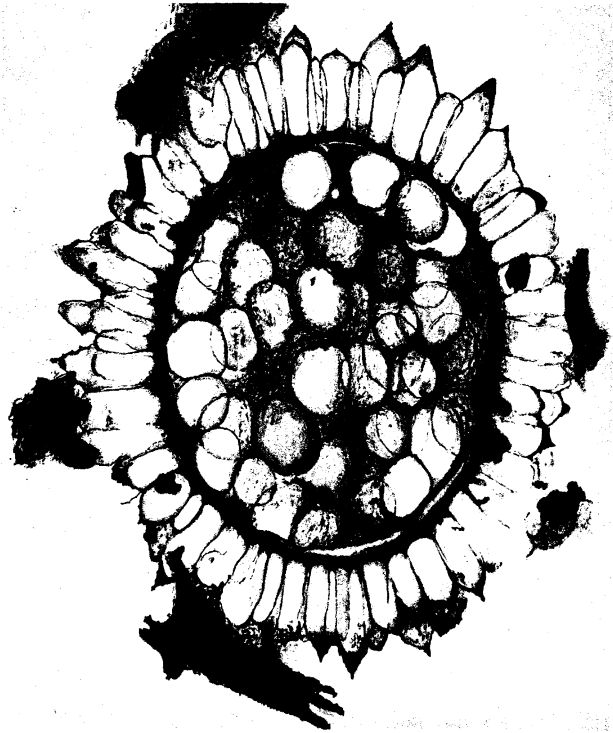
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A



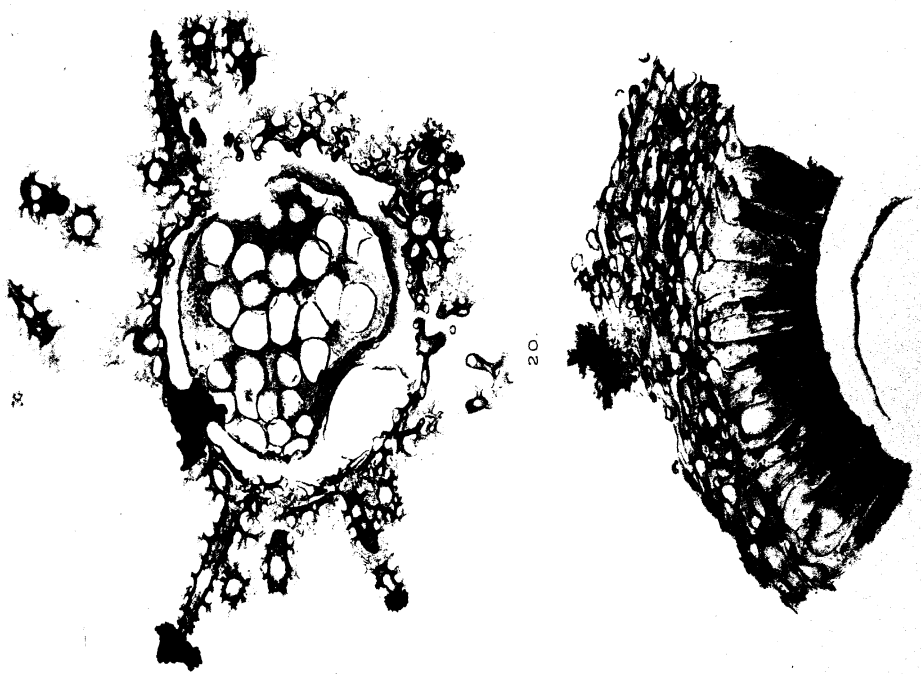
17.

Bath London.



18.

MELANOSPOROCARPON.



20.

Some Experiments on the Action of Wood on Photographic Plates.

BY

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With Plate XI.

DR. RUSSELL¹ discovered that certain woods have a definite action in darkness on a photographic plate. The present investigation was undertaken in order to ascertain whether this action provides a suitable method, first, for determining the incidence of decay before it is macroscopically visible; second, for observing the effect of exposing wood to various temperatures and degrees of moisture and its bearing upon the means of recognizing wood that has been kiln-dried; third, for the identification of timbers.

The interesting behaviour of the Pines in this connexion has been pointed out by Russell. He found in Scotch Pine, for example, that the light rings, i.e. the spring wood, have acted on the plate, giving dark rings, and that the autumn wood is without any action. All the Pines he investigated agreed with this, whilst the Larch gave a reverse picture, having the spring wood inactive and the autumn wood active. Professor Brereton Baker has proved that this action on the photographic plate is due to hydrogen peroxide given off from the wood. Experiments made during the course of this investigation show that there is no question of enzyme action, since steaming at 100° C. does not permanently destroy the activity of the wood.

In some woods, such as bass (lime) wood, Dr. Russell points out, a picture is formed which is not visible to the naked eye nor when a lens is used. It seemed possible, therefore, that this method might be a means of identification of timber and also might reveal the first sign of decay, making it easy to adopt remedial measures at an early stage.

METHOD.

The method employed was to place a specimen of wood on a photographic plate in the dark, usually with the smooth planed transverse surface

in contact with the plate, and secure it in place by means of a rubber band. It was enclosed in black paper and then placed in a cardboard box. It was found necessary to take further precautions against any light filtering through by placing the boxes in a tin, blackened on the inner surface and possessing a tight-fitting lid. After numerous experiments it was decided that, for the seasoned timber used, process plates were the most satisfactory, so Ilford process plates were used throughout. The most reliable developers were pyro soda made up without any bromide and Imperial hydrokinone. I am inclined to think the latter gave the best results, and it was the one used in the most critical work.

The optimum temperature and time of exposure were ascertained by preliminary experiments on Oak. The first plates were failures; in these cases air-dried oak was used and it did not give more than a faint negative. A plate was then prepared with two pieces of oak, one of which had been left in a moist atmosphere at 56°C . for forty-two hours and the other one was air-dry. The former gave a sharper image with greater density. It appeared that a certain amount of moisture was necessary to produce a good negative, so wherever air-dried material was used it was first exposed to moist air for times varying from 1 to 3 days. The factors of time and temperature of exposure interact. Generally speaking, the higher the temperature the shorter the exposure required, e.g. a good negative was obtained with oak in 72 hours at 30°C .; the same material gave as good a result when the exposure was 24 hours at 40°C . At 50°C . an exposure of 5 hours was sufficient, but the film was slightly injured. On the whole an exposure of 24 hours at 40°C . was found satisfactory.

Oak leaves an exact picture of itself on a photographic plate in the dark. The spring wood is inactive, giving a light band, and the autumn wood is active, giving a dark band showing the tracheides and fibres and parenchyma; the medullary rays are also clearly visible as dark lines. Fig. 1 shows a print of such an image, giving the positive. For convenience it is proposed to call the wood that acts on a photographic plate, giving a dark band, positive, and the wood that fails to act, so producing a light band, negative. In the oak, then, the spring wood is negative and the autumn wood is positive. In order to be quite certain of the interpretation of these positive and negative bands, two thin paper indicators were gummed on to the wood, one on a ring of spring wood and one on a ring of autumn wood. The wood does not act through the paper, so an active ring of wood produces a dark band on the film interrupted by a white line in the position of the paper. A comparison of the positions of the indicators on the wood and the negative soon determines whether it is the spring or autumn wood that is positive. Images taken without this precaution are apt to be misleading.

I. The Incidence of Decay.

The timbers used for these experiments were Oak and Pine in a fairly advanced stage of decay, since it was evident that, if no differences occurred between plates from sound and diseased timbers at that stage of decay, then it was unlikely that disease in an early stage could be detected. In all these cases the wood used was already air-dried, so as a preliminary each piece was exposed to moist air for three days. The specimens employed had a transverse surface not greater than $2\frac{1}{2} \times 3\frac{1}{2}$ in. and were finally placed on Ilford process plates of that size. Controls of normal Oak and Pine were photographed by the same method at the same time. No differences could be found in the images obtained, except where the disease had progressed so far that the wood was almost powdery. Even then the image partook of the same character in both, e.g. in Scotch Pine the normal wood gave spring wood positive and autumn wood negative. The same occurred in the diseased wood except for a blurring effect where the decay was very advanced. It was soon evident from these results that this method would not be successful for detecting disease before it is macroscopically visible.

II. The Effect of Varying Conditions of Temperature and Moisture and the recognition of Wood that has been kiln-dried.

Samples of Scotch Pine and Oak were dried in air, over sulphuric acid, over calcium chloride, and by heating to 100°C . for two days. Their weights were taken and negatives obtained in the dark. Then the samples were placed in moist air for three days, again weighed and images obtained in the dark. In the end each was dried at 100°C . for several days till the dry weight was obtained, and from these results the percentage of moisture was calculated.

SAMPLE I. *Scotch Pine.*

Condition.	Weight in grm.	Dry wt.	Mois- ture %.	Temp. of Exposure.	Time of Exposure.	Spring Wood.	Autumn Wood.	Condition of Negative.
1 a. Air dry	32.897	29.04	13.3	40°C .	24 hrs.	+	—	Fair
b. Moist air 3 days	36.275	29.04	24.9	"	"	+	—	No difference from 1 a
2 a. Dried over H_2SO_4	32.209	29.45	9.3	"	"	+	—	Better than 1 a
b. Moist air 3 days	35.085	"	19.0	"	"	+	—	As in 2 a.
3 a. Dried over CaCl_2	33.030	29.765	10.3	"	"	+	—	Good
b. Moist air 3 days	34.660	"	16.4	"	"	+	—	Not quite so intense as 3 a
4 a. Dried at 100°C .		52.44		"	"	+	—	Good
b. Moist air 3 days	56.981	"	8.6	"	"	+	—	" Better definition than 4 a

SAMPLE II. *Scotch Pine.*

Condition.	Weight in grm.	Dry wt.	Moisture %	Temp. of Exposure.	Time of Exposure.	Spring Wood.	Autumn Wood.	Condition of Negative.
1 a. Air dry	31.485	27.860	13.0	40° C.	24 hrs.	+ ?	- ?	Very faint
b. Moist air 3 days	35.935	"	25.7	"	"	+	-	More dense than 1 a
2 a. Dried over H ₂ SO ₄	31.549	28.880	9.1	"	"	+	-	Fair
b. Moist air 3 days	34.760	"	20.3	"	"	+ ?	- ?	Faint
3 a. Dried over CaCl ₂	30.499	27.565	10.68	"	"	+	-	Good
b. Moist air 3 days	32.335	"	17.35	"	"	+	-	" (a little clearer)
4 a. Dried at 100° C.		29.279		"	"	+	-	Better than any
b. Moist air 3 days	33.190	"	13.35	"	"	+	-	As good as 4 a

The results from the Oak used were on the same lines as these. It is obvious from these results that the amount of moisture present seems to affect the kind of negative produced very little, if at all. The negative obtained from wood that has been dried at 100° C. and that from the same piece of wood containing 13.35 per cent. of moisture are indistinguishable. In each case the negative obtained from an air-dried piece of wood, exposed at a given temperature and for a given time, is fainter than where the wood has been dried over calcium chloride or at a temperature of 100° C. This difference is hardly stable or marked enough to use it as a criterion for determining the exact temperature used to season a given timber or the amount of moisture present in a given timber.

Images were obtained from kiln-dried Scotch Pine and Oak by this method, and they agreed in every detail with the negatives obtained from air-dried specimens which had been left in moist air for three days. The only difference observable in some of the samples was that wood, dry heated to 100° C., often gave a denser image with a shorter exposure than was the case with the unheated samples, but the character of the image was the same. Dry heating to 100° C. may be said to increase the activity of the wood.

The effect of heating the wood in steam was observed. Thin pieces of four samples of Scotch Pine were subjected to steam for twelve hours. Images were then obtained in the dark. Three of the samples made no impression on the plates after an exposure at 40° C. for forty-eight hours. The fourth gave a fair negative, but rather poor in intensity. A longer exposure was tried with the same result. The steaming had evidently inhibited the activity of the wood; controls showed activity in all four cases. After forty-one days images of the samples were again taken.

They were exposed at 40° C. for 120 hours. In every case a good negative was obtained. It would appear that steaming has the effect of diminishing the activity of the wood on a photographic plate for a time only and not permanently. This method is, therefore, useless for detecting artificial seasoning or drastic treatment with wet or dry heat.

III. The Identification of Timbers.

Russell said in his papers that Scotch Pine in the dark acts on a photographic plate in such a way that the spring wood gives a dark ring and the summer or autumn wood does not have any effect. So the plate shows a dark ring for every ring of spring wood and a light one for every ring of autumn wood (Fig. 2). He stated that all the Pines he tested behaved in the same way. On the other hand, Larch gives a reverse image, the spring wood being negative and the autumn wood positive (Fig. 3). It seemed that this method would provide a means of distinguishing between a Pine and a Larch.

In the course of my preliminary experiments with Scotch Pine, to determine time of exposure and optimum temperature, I came across a specimen of Scotch Pine that gave the reverse image; its spring wood was negative and autumn wood positive, so it resembled a Larch. Structural examination confirmed that it was Scotch Pine. It was then necessary to ascertain the action on a photographic plate in the dark of a number of different specimens of Scotch Pine, under varying conditions, to see if there were other exceptions. Also it was considered that there might be some difference in behaviour of heart-wood and sap-wood, so in the specimens selected for experiment some were pure sap-wood, some pure heart-wood, and some partly one and partly the other. In each case the history of the particular Scotch Pine was known and two pieces of wood were selected from each specimen. One piece was air-dried, so it was left in moist air before the experiment was performed; the other had been dried at 100° C. for two days.

The following results were obtained:

<i>No. of Scotch Pine.</i>	<i>No. of Plate.</i>	<i>Condition of Wood.</i>	<i>Temp. and Time of Exposure.</i>	<i>Type of Wood.</i>	<i>Spring Wood.</i>	<i>Autumn Wood.</i>
321 Fig. 4	52	Moist air 3 days	40° C. 24 hrs.	Sap-wood chang- ing to heart- wood	+	—
	56	Dried at 100° C.	" "	Do.	+	—
	100	Do.	27° C. 72 hrs.	Do.	+	—
	114	Moist air 3 days	40° C. 24 "	Do.	+	—
	321	Do.	" 48 "	Do.	+	—
	131	Dried at 100° C.	" 96 "	Do.	+	—
	147	Moist air 1 day	" 48 "	Do.	+	—
	191	Steamed 12 hrs. and left 41 days	" 120 "	Do.	+	—

<i>No. of Scotch Pine.</i>	<i>No. of Plate.</i>	<i>Condition of Wood.</i>	<i>Temp. and Time of Exposure.</i>	<i>Type of Wood.</i>	<i>Spring Wood.</i>	<i>Autumn Wood.</i>
220	49 A	Moist air 3 days	40° C. 24 hrs.	Mostly heart-wood	+	—
	58	Dried at 100° C.	" 24 "	Do.	+	—
	101	Do.	27° C. 72 "	Do.	+	—
	135	Moist air 3 days	40° C. 96 "	Do.	+	—
	140	Dried at 100° C.	" 21 "	Do.	+	—
	161	Do.	" 24 "	Do.	+	—
	192	Steamed 12 hrs. and left 41 days	" 120 "	Do.	+	—
260	30	Dried at 100° C.	30° C. 72 hrs.	Heart-wood with small sap-wood at one edge	+	—
	42	Moist air 3 days	50° C. 21 "	Do.	+	—
	44	Dried at 100° C.	" 24 "	Do.	+	—
	45	Do.	" 5 "	Do.	+	—
	111	Moist air 3 days	40° C. 120 "	Do.	+	—
	128	Dried at 100° C.	" 96 "	Do.	+	—
	148	Moist air 3 days	" 48 "	Do.	+	—
482	55	Dried at 100° C.	40° C. 24 hrs.	Sap-wood	+	—
	109	Do.	" 72 "	Do.	+	—
	482	Moist air 3 days	" 108 "	Do.	+	—
	134	Do.	" 96 "	Do.	+	—
	138	Dried at 100° C.	" 21 "	Do.	Activity all over — but some ac- tivity	
	159	Do.	" 24 "	Do.		
	190	Steamed 12 hrs. and left 41 days	" 120 "	Do.	+	—

In the foregoing cases, whatever the time or temperature of exposure or condition of moisture of the specimen, the result shows that the spring wood is positive, giving a dark ring on the film, and the autumn wood is negative. These all agree with Dr. Russell's results. Other cases show exactly the reverse image :

<i>No. of Scotch Pine.</i>	<i>No. of Plate.</i>	<i>Condition of Wood.</i>	<i>Temp. and Time of Exposure.</i>	<i>Type of Wood.</i>	<i>Spring Wood.</i>	<i>Autumn Wood.</i>
191	50	Moist air 3 days	40° C. 24 hrs.	Sap-wood	—	+
Fig. 5	105	Do.	27° C. 72 "	Do.	—	+
	110	Do.	40° C. ?	Do.	—	+
	191	Do.	" 108 "	Do.	—	+
	156	Do.	" 96 "	Do.	— ?	+
	200	Do.	" 72 "	Do.	—	+
Fig. 6	54	Dried at 100° C.	" 24 "	Do.	+	—
	126	Do.	" 96 "	Do.	+	—
471	112	Moist air 3 days	40° C. 24 hrs.	Heart-wood	—	+
Fig. 7	136	Do.	" 96 "	Do.	—	+
	195	Do.	" 96 "	Do.	—	+
	218	Do.	" 144 "	Do.	—	+
	224	Do.	" 120 "	Do.	—	+
	107	Dried at 100° C.	" 24 "	Do.	+	—
	127	Do.	" 96 "	Do.	+	—

<i>No. of Scotch Pine.</i>	<i>No. of Plate.</i>	<i>Condition of Wood.</i>	<i>Temp. and Time of Exposure.</i>	<i>Type of Wood.</i>	<i>Spring Wood.</i>	<i>Autumn Wood.</i>
229	137	Moist air 3 days	40° C. 21 hrs.	$\frac{3}{4}$ sap-wood, } $\frac{1}{4}$ heart-wood }	—	+
	158	Do.	" 96 "	Do.	+	—
	201	Do.	" 24 "	Do.	—	+
	139	Dried at 100° C.	" 21 "	Do.	So active that no detail seen	
	145	Do.	" 6 "	Do.	Slightly +	+
	160	Do.	" 24 "	Do.	" +	+
283 Fig. 8	283	Moist air 3 days	40° C. 48 hrs.	$\frac{1}{2}$ sap-wood, } $\frac{1}{2}$ heart-wood }	+	—
	154	Do.	" 96 "	Do.	+	—
	193	Steamed 12 hrs. and left 41 days	" 120 "	Do.	+	—
Fig. 9	123	Dried at 100° C.	" 72 "	Do.	—	+

In these cases specimens of Scotch Pines No. 191 and 471 gave throughout the spring wood negative and the autumn wood positive, as in the Larch, where air-dried timber was used. In these two cases also, where the timber has been subjected to 100° C. for two days, its activity is totally altered; the spring wood becomes positive and the autumn wood negative, just as in the specimens given in the first tables. Specimen 229 gave rather puzzling results, since plate 158 apparently contradicts the other negatives obtained. Both plates 158 and 201 are clear and definite negatives, though the position of the indicators is much better defined in the latter. The piece of specimen 229 which had been dried at 100° C. for two days showed activity all over, so that a very short exposure had to be used to obtain any differentiation. Even then the spring wood was still slightly active, though not so active as the autumn wood. This, then, is inclined to the Larch picture. Specimen 283 shows the Larch image in the case of wood dried at 100° C. for two days, the spring wood being negative and the autumn wood positive, whilst the air-dried wood of 283 follows the usual trend for Scotch Pines (see Figs. 8 and 9).

These results cannot depend on the time and temperature of exposure or the amount of moisture present, since these varied without any corresponding variation in the image. The type of wood used—sap-wood or heart-wood—does not affect the results, for the specimens which gave results consistent with those of Dr. Russell were some heart-wood, some sap-wood, and some both. In the same way Scotch Pines Nos. 471 and 191, which were consistent in producing the reverse image, were one heart-wood and the other sap-wood.

It was also discovered that if the same piece of wood was used many times on photographic plates its activity diminished, so that a piece of wood which gave a perfectly good negative at 40° C. in 24½ hours at first required a time of exposure of 3-4-5 days later on to obtain anything like the same intensity.

The Scotch Pines that gave reverse images were examined with special reference to the distribution of resin ducts, since it was thought that some difference in the number of resin ducts in the autumn wood might account for the reversal. However, it was found that numerous resin ducts were present in the autumn wood of the heated and unheated pieces of all the specimens used, whether they gave the ordinary or reverse image. Whatever the cause of this reversal of image in some Scotch Pines, there is no doubt of its occurrence, and it certainly weakens the case for using this method for identification purposes. Images of Larch were obtained in the same way. Four specimens were chosen and two pieces taken of each, dried at 100° C. and air-dried. In these cases the spring wood was negative and the autumn wood positive in each one. It would be necessary to use this method with many more specimens before it could be deduced that a reversal never takes place in the Larch.

The same method was used with examples of timber from other Coniferae, with the result that they were found to fall into two categories:

Spring wood + and Autumn wood - Spring wood - and Autumn wood +

Pinus sylvestris (usually)	Larix
„ cubensis	Pseudotsuga Douglasii
„ palustris	„ Taxifolia
„ mitis	„ macrocarpa
„ Khasia	Picea
„ longifolia	Abies
„ Merkusii	Agathis Australis
„ Pinaster	Podocarpus Totara
	Cupressus Lawsoniana
	Pinus Strobus
	„ excelsa
	„ Lambertiana
Pinus Gerardiana	

These results show that the Coniferae can be divided into two groups according to their type of activity on a photographic plate. An examination of these two groups shows that the first consists of Pines, which all show certain morphological features. That is, they all have diploxylic needles; their leaf-sheaths are not deciduous; their cone-scales have a central umbo each, and the ray-tracheides bear denticulations on their walls. These Pines also agree in producing an image on a photographic plate in which the spring wood is positive and the autumn wood negative.

The second group consists of other Conifers and that class of Pines that have haploxylic needles, their leaf-sheaths deciduous, their cone-scales each with a terminal umbo, and the ray-tracheides bearing no denticulations

on their walls. This group agrees in producing an image on a photographic plate in which the spring wood is negative and the autumn wood positive.

*Pinus Gerardiana*² is very interesting in this connexion. It has haploxylic needles with deciduous leaf-sheaths; in these features it agrees with the second group. On the other hand, it is a three-needled species with a central umbo on its cone-scales. The ray-tracheides bear very scantily and feebly suggested denticulations; these features agree with the first group. *Pinus Gerardiana* is, then, intermediate, morphologically, between the two groups. The result by this photographic method is peculiarly interesting in view of this. The image obtained showed great activity all over, with the medullary rays particularly marked in dark lines. The general activity is so great that it is somewhat difficult to distinguish between the spring and autumn wood, the latter being, if anything, slightly more active. This photographic method leaves *Pinus Gerardiana* occupying an intermediate position between the two groups, in agreement with its morphological position.

CONCLUSIONS.

1. The action of wood in darkness on photographic plates does not give a suitable method for discovering the incidence of decay before it is macroscopically visible.
2. Neither does it form a sufficiently critical method of determining the amount of moisture present in timber nor of recognizing kiln-dried timber or drastically heated timber.
3. The case for identification of timber by this method is somewhat weakened by the fact that different samples of Scotch Pine may give reverse results. On the other hand, apart from these few cases of reversal in Scotch Pine, it has been found that this photographic method confirms the division of the Pines into two groups already differentiated by their morphological features.

In conclusion, my best thanks are due to Professor Percy Groom, M.A., D.Sc., at whose suggestion this investigation was undertaken, for the material he put at my disposal and for his helpful criticism; also to Professor Brereton Baker, F.R.S., for his information on the chemical action involved. This research was subsidized by the Department of Scientific and Industrial Research.

June, 1921.

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EXPLANATION OF PLATE XI.

Illustrating Miss Williamson's paper on Some Experiments on the Action of Wood on Photographic Plates.

Fig. 1. Print from negative from sample of Oak, showing the spring wood dark, autumn wood light, and medullary rays light. This is the reverse of the negative.

Fig. 2. Specimen 260, Scotch Pine, which had been subjected to 100° C. for two days some months previously. The negative gave the spring wood dark and the autumn wood light, so the reverse is shown in the print.

Fig. 3. Larch. The negative gave spring wood light and autumn wood dark, so in the print there are broad dark rings in the position of the spring wood and narrower light bands in the position of the autumn wood. Cf. Fig. 2.

Fig. 4. Scotch Pine, Specimen 321.

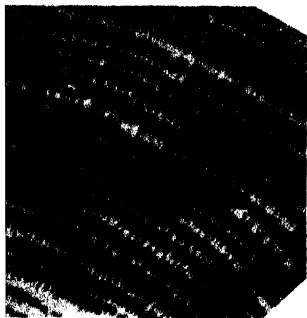
Fig. 5. Scotch Pine, Specimen 191. This specimen showed broad bands of both spring and autumn wood. The paper indicators showed that this gave a negative of the Larch type. Cf. Fig. 3.

Fig. 6. Scotch Pine, Specimen 191, which had been subjected to 100° C. for two days some months before the negative was obtained. This follows the general rule for Scotch Pine, as in Fig. 2, and is the reverse of Fig. 6.

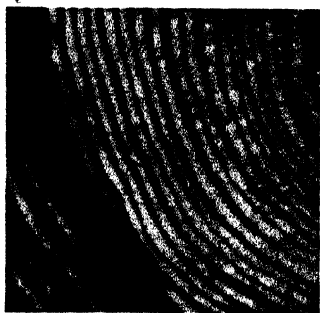
Fig. 7. Scotch Pine, Specimen 471, air-dried. This is again the Larch type of image, as in Fig. 3.

Fig. 8. Scotch Pine, Specimen 283, air-dried. This follows the normal rule for Scotch Pines—spring wood active and autumn wood inactive.

Fig. 9. Scotch Pine, Specimen 283, previously heated to 100° C. for two days. This shows a negative with spring wood inactive and autumn wood active.



1.



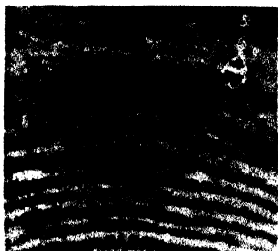
2.



3.



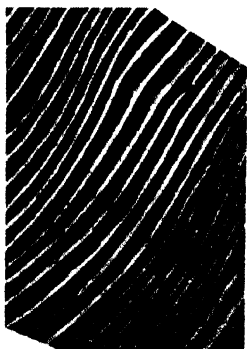
4.



9.



6.



7.



8.



5.

Huth coll.

Studies in the Physiology of Parasitism.

VIII. On the Exosmosis of Nutrient Substances from the Host Tissue into the Infection Drop.

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With one Figure in the Text.

IN previous papers of this series¹ it was shown that the fungus *Botrytis cinerea*, though possessing an active cytolytic principle, was unable to act upon the cells of the host tissue so long as an intact cuticle separated it from them. Evidence was brought forward to show that the penetration of the cuticular membrane was effected by mechanical rupture, and it was only when this had taken place that the fungus was able to attack directly the host cells. So far the question had been treated from the point of view of the influence of the parasite upon the host. There still remained for examination the converse relation of host to parasite. For whereas, antecedent to penetration, the tissue of the host was found to be wholly unaffected by the presence of the parasite, it appeared not improbable that the host plant might be able to influence in some way the behaviour of the parasite. Such an influence might take the form of a stimulus to germination or of a chemotropic stimulus. The present paper deals with the former question, viz. with the passive exosmosis of nutrient material from the host cells through the cuticle.

The method of experiment generally was as follows: Drops of distilled water of a standard size (*c.* 0.05 c.c.) were laid on the surface of plant organs and allowed to remain for some time, generally for twenty-four hours. They were then removed and examined to see whether any change had taken place in them due to their having been in contact with the plant.

Two methods of examination were adopted:

- (1) Determination of the electrical conductivity of the fluid.
- (2) Determination of the effect of the fluid on the germination of fungal spores.

¹ Ann. Bot., vol. xxx, 1916, pp. 389, 399.

For the determination of conductivities, the method described by Blackman and Paine¹ was employed. The cell consisted of a fine pipette with small bulb blown near the pointed end, into which the platinum leads were fused. The bulb was of such a size that it was completely filled by the drop. By means of a rubber cap with screw adjustment on the distal end of the pipette, the position of the drops in the cell could be accurately controlled. The use of a constant temperature bath was dispensed with and the readings made at laboratory temperature. In all cases the temperature was noted so that correction of results could be effected. In view of the magnitude of the differences observed, the error due to slight variations in temperature could be ignored.

To determine the capacity of the drops to stimulate spore germination, they were placed on clean glass slides, and a drop of a suspension of spores in water was added to each. Drops of water which had lain for twenty-four hours on glass slides or which came directly from the stock of distilled water were similarly treated and served as controls. After a certain time the state of the drops as regards germination of the spores was determined. In this connexion a mere count of the percentage of germinated spores is of doubtful value, as it fails to distinguish between a case where a certain percentage of spores germinates feebly and one in which a similar percentage germinates vigorously. The figure obtained in this way may thus give no true representation of the germinative picture displayed. A more accurate method was found in the measurement, by means of a micrometer eye-piece, of the total length of germ-tube of a number of spores chosen at random. In this way the 'average germ-tube length' could be determined. In view of the amount of variation which obtains between individual spores, a large number of counts and measurements (25, 50, or 100, according to circumstances) was made in each case. This method is also incomplete, inasmuch as it fails to represent the difference between the thin feeble germ-tubes which are formed in water or very dilute nutrient and the stout vigorous ones formed in more concentrated nutrients, but it seemed to be the best available.

In general, each drop was examined according to both methods—that is, it was removed from the plant surface by means of the pipette and its conductivity determined. It was then placed on a glass slide, the suspension of spores added, and the amount of germination ensuing in a given time measured. In this way a duplicate series of results was obtained. The electrical method gives only a measure of the amount of dissociated electrolytes which have diffused out of the plant; for the purpose of determining the amount of nutrient material appearing in the drops, the spore-germination method obviously is the direct one. The degree of parallelism exhibited by the two series of results will be illustrated subsequently.

¹ Ann. Bot., vol. xxxii, 1918, p. 69.

The plant material consisted of leaves and floral structures, viz. : petals of *Cereus*, *Phyllocactus*, *Gloxinia*, *Lilium*, *Tulip*, *Rose*, *Begonia*, *Viola*, *Sweet-pea*, *Dahlia*, *Geranium*, *Cydonia*, *Pyrus*, and leaves of Broad Bean. A large amount of the work was carried out with the petals of *Cereus spectabilis*, which offers special advantages. These petals are highly and uniformly coloured, and lend themselves readily to plasmolysis studies by means of the microscope; from the same flower a large number of petals can be obtained which show similar behaviour, and which, especially when taken from the unopened bud, show a perfectly clean surface; the results obtained by both methods of experimentation are well defined; and lastly, the petals present a flat surface and are moderately easily wetted. The latter is an important practical point which may be illustrated by a few examples. In the case of such petals as those of *Viola* and of some varieties of *Rose*, the drops do not wet the petals at all. They maintain a more or less spherical shape, and from their appearance it is clear that a film of air is entrapped between them and the surface of the petal. The drop, therefore, has only very limited contact with the petal. In such cases it is found that the exosmosis figures are always very low. Such petals are further very troublesome in use, in that the drops are very liable to shift, coalesce, or run off. At the other extreme is the case of the Bean leaf, where the drops tend to spread too far, especially along the line of the veins, thus making it impossible to ensure that the drops occupy approximately uniform areas of the plant surface. In the case of *Cereus* petals an intermediate degree of wetting is shown; comparatively good contact obtains between drop and petal, and the drops present a fairly uniform appearance when laid on the petals.

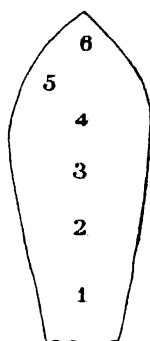
An attempt was made to get over this difficulty of non-wetting in the case of some plants by the addition of traces of substances such as Castile soap and saponin to the water, but the preparations tried were found to possess too high a conductivity for use in this connexion.

For the reasons given above petals of *Cereus* were selected for detailed examination. As was anticipated, a considerable degree of variation was obtained from drop to drop. Certain well-marked rules were, however, made out, of which the following is an account.

On the same petal the conductivity of the drops varies from place to place, being smallest in the centre along the line of the midrib, and increasing towards the distal (free) end of the petal. The greatest values are obtained along the margins, and especially near the tip. The following figures, representing the conductivity of drops which have lain in contact with the petals for twenty-four hours in a moist Petri dish at laboratory temperature (17–20° C.), illustrate this point. The numbers in the diagram (see next page) indicate the position of the drops on the petal.

(Conductivity of $m/100$ KCl at $20^{\circ} = 72.7$.¹ Conductivity of the distilled water varied within the limits 0.8–1.2.)

Drop.	Petal I. Conductivity.	Petal II. Conductivity.
1	2.2	4.0
2	2.2	5.4
3	2.4	5.1
4	5.8	7.4
5	7.6	10.3
6	8.5	19.1



In addition to observing that this rule held throughout all the work with *Cereus*, it was found in two experiments specially set up to determine this point that the average conductivity of 58 central drops was 4.7 (min. 2.2, max. 11.2), and that of 29 marginal drops was 15.2 (min. 6.0, max. 39.4).

The amount of exosmosis from the same petal in a given time increases day by day. This was shown by adding new drops to the same places each day after the drops of the preceding day had been removed and examined. In such an experiment as this an appreciable risk of contamination of the drops by fungal spores is incurred, and a certain number of the petals after some days showed discoloured spots which were found to be caused by spores of *Botrytis*. Such petals were discarded. The following figures give the average conductivity (av. of 19 drops) obtained on three consecutive days from petals of *Cereus* which showed no evidence of fungal contamination on the fourth day.

	1st day.	2nd day.	3rd day.
Average conductivity.	3.6	7.8	18.6

The same type of result was obtained with petals and sepals of *Tulip*. In this case the rise of rate of exosmosis with time was found to be more gradual.

The effect just described is due partly to ageing of the plant structure and partly to the fact that the method of treatment results in better wetting of the petals as time goes on. Experiments in which one batch of petals was subjected to the above day-by-day treatment, while a control batch was kept for the same time without the treatment, showed that the latter gave considerably higher figures than had been given at the commencement of the experiment, but not so high as those which were being given by the

¹ In arbitrary units.

treated petals. There is thus a distinct ageing effect (as was to be expected). The wetting effect is readily observable. It was seen that the earlier drops laid on a given spot were, on application of the pipette, readily sucked up in their entirety, whereas in the case of the later drops a film of liquid was left on the surface of the petal. Whenever such a degree of wetting was reached, i. e. such that it was impossible to pick up the complete drop with the pipette, high exosmosis figures were invariably obtained.

The presence of pollen-grains in the drops gives rise to high conductivity figures. This is shown by the following experiment with *Cereus* petals. The petals were washed (without rubbing) in a stream of water, rinsed with distilled water, then allowed to dry, after which drops of distilled water were placed on each. To a certain number of these a quantity of *Cereus* pollen was added on the point of a platinum wire. After twenty-four hours the conductivity of the drops was measured, with the following results :—

Pollen-free drops.	Average conductivity after 24 hours 11 drops ¹ = 3.3 (min. 2.8, max. 4.2)
Pollen-containing drops.	Average conductivity after 24 hours 16 drops = 73.8 (min. 54.6, max. 92.3)

It should be stated that the pollen-containing drops show a greater tendency to spread over the surface of the petal than the pollen-free ones. This in itself, as giving rise to a greater area of contact with the petal, would tend to cause the conductivity figures to be higher in the former case. The disparity in this respect, however, would account for only a very small portion of the difference in conductivity observed. Similar results were obtained with pollen of Rose and Lily, and it is highly probable that they apply to pollen generally.

In determining the amount of exosmosis from the petal itself it is therefore necessary to remove completely any pollen that may be present. This can be effectively done in the manner described above.

Incidental to this necessity for the removal of pollen, it was found that mechanical rubbing, even though gentle, considerably increased the conductivity figures obtained. Thus in one experiment with petals of *Cereus* the average conductivity of drops laid along the midrib of washed and slightly rubbed petals was 12.8, whereas that of drops laid on a similar series of petals (alternating in the flower with the preceding) which had simply been rinsed amounted to only 5.0 (19 drops in each case). In the case of the rubbed petals it was observable that better contact between the petal and the drop had been established. The effect is undoubtedly due to the removal of the surface bloom of the petals by the mechanical treatment.

Throughout this work strict attention was paid to the question as to whether the experimental petals remained alive during the treatment. In petals generally, and in *Cereus* in particular, death is shown macroscopically

by the petal becoming limp and flaccid and by the discharge of the colour. Microscopically it is shown by the failure of the cells to undergo plasmolysis in hypertonic solutions. In no case were results taken from petals which showed any discolorations due either to the accidental presence of fungal contamination or to the general ageing of the petals. Numerous tests were made on the petals at the end of each experiment to see if any diminished capacity for plasmolysis could be demonstrated. In this way it was shown that petals which had been under treatment for several days still possessed, as far as could be seen, a capacity of undergoing plasmolysis equal to that of fresh ones. In both cases, after immersion of the petals in molar potassium nitrate, a very large percentage of distinctly plasmolysed cells, i. e. with sharply marked contracted highly coloured vacuoles, is seen, together with a small percentage in which the evidence of plasmolysis is not so distinct. Numerous tests led to the conclusion that the treated and the fresh petals were indistinguishable in their plasmolytic features. It was frequently found that the experimental petals showed after several days' treatment a characteristic mottling due to the presence of numerous small translucent patches. Examination of the latter showed that they were due to injection of the intercellular spaces with liquid. The distribution of these injected patches showed no relation to the position that had been occupied by water-drops, whence it follows that the liquid was derived from the cells themselves. Such self-injected petals invariably gave high conductivity figures. Nevertheless, on immersion in molar potassium nitrate they showed a degree of plasmolysis indistinguishable from that of fresh petals.

GERMINATION STUDIES.

The conductivity method was largely used on account of its ease and simplicity, but, as was pointed out above, the real criterion of the importance of exosmosis in the physiological processes of infection is the effect of the fluid of the experimental drops on fungal germination. Germination studies, carried out as already described, showed that a high conductivity figure was associated with high germinative capacity and vice versa. The degree of correspondence observed will be seen in the following table, which embodies the results obtained in an experiment specially set up for this purpose.

In this experiment petals were obtained from an unopened bud of *Cereus*. They were thus free from fungal contamination or other blemish. In order to obtain a wide range of conductivity figures, the water drops were allowed to remain in contact with the petals for different lengths of time.

Drop.	Conductivity.	Average length of germ tube (in micrometer divisions—av. of 25 measurements).			
		12 hrs.	16 hrs.	24 hrs.	36 hrs.
Distilled water	0.8	0.16	0.22	0.20	0.46
Tap-water	48.0	0.00	0.00	0.00	—
Drops on Cereus petal	1.7	0.22	0.24	0.58	0.80
" "	3.5	0.60	1.46	2.32	—
" "	4.5	1.00	—	3.32	3.48
" "	4.8	0.76	1.66	2.60	2.92
" "	4.9	0.70	—	2.44	2.80
" "	8.4	2.16	3.00	5.00	—
" "	8.6	1.50	2.84	4.28	—
" "	9.4	2.02	—	4.28	5.16
" "	9.5	2.04	2.72	3.88	5.12
" "	11.3	1.84	2.64	3.92	6.12
" "	14.2	2.24	—	5.88	8.6
" "	22.7	2.92	—	6.8	10.20
" "	26.1	2.48	5.12	6.10	10.20
" "	27.4	2.52	5.04	6.10	10.20
" "	32.0	3.00	—	6.10	10.20
" "	32.1	2.72	—	—	10.20
" "	59.5	2.88	5.60	6.10	10.20

The conductivity figures are arranged in ascending series. It will be seen that the germination figures increase in the same direction, though with small irregularities. When one remembers the variation in germinative capacity among spores and the necessity of carrying out the measurements of germ-tube length rapidly, and therefore over a comparatively small number of spores (in this case 25), these irregularities are not surprising.

In the following table the conductivity figures are grouped within certain limits and the corresponding amounts of germination averaged. In this table it will be seen that the irregularities have almost completely disappeared.

Drop.	Conductivity.	Average length of germ tube.			
		12 hrs.	16 hrs.	24 hrs.	36 hrs.
Distilled water	0.8	0.16	0.22	0.20	0.46
Drops on Cereus petal	1-2	0.22	0.24	0.58	0.80
" "	2-5	0.76	1.56	2.67	3.07
" "	5-10	1.93	2.85	4.36	5.14
" "	10-20	2.04	2.64	4.90	7.30
" "	20-35	2.73	5.08	6.10	10.20
" "	> 35	2.88	5.60	6.10	10.20

In a similar experiment the drops which had lain on Cereus petals were compared as regards both conductivity and germinative capacity side by side with dilutions of a turnip extract. The figures are contained in the following table :

Drop.	Conductivity.	Average length of germ tube.		
		10 hrs.	20 hrs.	40 hrs.
Distilled water (av. of 3)	0.70	0.44	0.73	1.02
From glass slides	1.19	0.44	0.83	1.02
From <i>Cereus</i> petals	1-2 av. 1.50	0.64	1.24	2.20
" "	2-3 av. 2.15	0.67	1.74	2.71
" "	3-4 av. 3.71	1.22	3.23	5.28
" "	4-5 av. 4.28	1.67	3.27	5.94
" "	5-6 av. 5.60	2.04	6.08	7.52
Turnip extract 1/1	73.0	0.64	6.08	weft ¹
" " 1/5	61.6	2.88	> 10	"
" " 1/25	38.8	4.20	> 10	"
" " 1/100	16.8	4.28	> 10	"
" " 1/400	6.22	3.76	> 10	"
" " 1/1,000	3.28	3.96	7.48	"
" " 1/4,000	1.40	3.76	lost	"
" " 1/20,000	1.14	2.80	4.28	5.48
" " 1/100,000	0.77	1.64	3.20	5.28

¹ The compactness of the wefts was seen to be in order diminishing with dilution. The amount of growth was obviously greatest in the full strength extract.

It will be noted that the amount of germination on drops from *Cereus* petals is considerably less than that in turnip extract of the same conductivity. Similar dilutions of apple extract showed the same feature. This is no doubt to be ascribed to the fact that the turnip and apple extracts contain the bulk of the soluble cell contents, both those which are readily and those which are more slowly diffusible; whereas the drops from *Cereus* petals contain a preponderating proportion of the more readily diffusible constituents such as salts. The presence of these would raise the conductivity of the drops, while the smaller proportion present of the more slowly diffusible carbohydrates, &c., would have the effect of making the fungal germination meagre.

Incidentally it may be seen that, as the concentration of turnip extract increases, the amount of germination passes through a maximum. This is well shown in the figures for the germination after 10 hours. It is still shown to some extent after 20 hours, but has disappeared in 40 hours, when the amount of growth is greatest in the strongest extract. This feature is shown by other extracts and by other fungi than *Botrytis*.

The correspondence between the conductivity and the germinative capacity of the drops from *Cereus* petals is again clearly shown in the above table.

It will be noticed that the conductivity of drops laid on glass slides is greater than that of the original distilled water freshly taken from the stock bottle. A similar small increase in conductivity was also shown by drops laid on clean quartz slides and on glass slides maintained in a vacuum. The cause of this slight gain in conductivity was not clearly made out. It is probably due to a variety of causes, partly to solution of carbon dioxide from the air, partly to leaching of salts from the glass, and partly to accidental contamination with specks of dust. From the point of view of

the investigation it is of no importance, as the slight gain in conductivity is not accompanied by any increase in the capacity to stimulate germination.

The failure of *Botrytis* spores to germinate in the tap-water (hard) is worthy of note.

The correspondence between conductivity and amount of germination was shown also for drops laid on petals of Poppy, Iris, Petunia, Geranium, and Rose.

EXOSMOSIS FROM OTHER PLANTS.

The results as detailed in the case of *Cereus* are paralleled in the case of other plants. A brief summary of the type of results obtained may be given here.

Petals of *Phyllocactus* gave figures for conductivity and germinative capacity of about the same magnitude as those of *Cereus*. They are, however, much inferior to the latter from the point of view of infection studies.

Petals of Tulip (var. 'Keizer Kroon'), Cydonia, Sweet-pea, Petunia, and Geranium give in general smaller conductivity figures. The effect on germination is, however, quite distinct.

Petals of Rose, Viola, *Lilium*, Begonia, Gloxinia, and Tulip (var. 'Darwin') give low conductivity figures and only very slight effect on germination.

The last-mentioned petals have the common feature of being difficult to wet. This is specially marked in petals of Viola, where the drops maintain an almost spherical shape and can hardly be said to be in contact with the petals at all. In the case of Begonia (female flower) it was noticed that drops on the petals showed very incomplete wetting with low conductivity and low germination figures, whereas on the ovary wings much better wetting took place, with a corresponding increase in the figures obtained for conductivity and germination. The same applied to the lobes of Gloxinia petals as compared with parts of the corolla lower down. It is highly probable that if a suitable method could be found for overcoming this difficulty of non-wetting it would be found that the amount of exosmosis and the corresponding capacity of the drops to stimulate spore germination would in all cases be considerably increased.

EXOSMOSIS IN RELATION TO ATTACK.

Some experiments will now be described in which the fact of exosmosis of nutrient into the infection drop is brought out by an examination of the progress of attack. This method is based on a comparison of the time required to effect attack when the spores are sown in water with that in the case in which the spores are sown in nutrient extract.

The effect of the addition of extraneous nutrient to the infection drop is to accelerate the incidence of attack (e.g. attack of *Botrytis* on petals of Rose, &c.), or in extreme cases to bring about attack where no attack takes place in its absence (e.g. attack of *Botrytis* on leaves of Broad Bean). In the case of a plant which allows a considerable amount of nutrient to diffuse into the infection drop, one would anticipate that the time required for spores sown in water to produce infection would approximate to that required for spores sown in nutrient extract, whereas the difference in time would be more marked in the case where less nutrient was allowed to pass out.

An experimental difficulty is met with in the fact that *Botrytis* spores of a certain type are able to germinate to some extent in pure water, and it is highly probable that this degree of germination is sufficient to enable them to attack delicate structures such as petals even without the addition of any nutrient to the infection drop. In order to make the results more sharply marked, measures were therefore taken to suppress the germination of the spores in pure water.

The first method depends on the fact that the germinative capacity of *Botrytis* spores is reduced by age. Thus, while a suspension of young spores (10 days from sowing of cultures) will readily germinate in pure water, those from a six-weeks old culture will no longer do so, though they are quite capable of germination in nutrient. Using spores of these two types the following results were obtained in comparative infection experiments with *Cereus* (considerable exosmosis) and *Gloxinia* (small exosmosis) :

<i>Type of spore.</i>	<i>Sown in.</i>	<i>Plant.</i>	<i>Time for infection in all the drops.</i>
Young	Turnip extract	<i>Cereus</i>	10-11 hours
"	Water	"	11-12 "
Old	Turnip extract	"	20-23 "
"	Water	"	22-26 "
Young	Turnip extract	<i>Gloxinia</i>	13-16 "
"	Water	"	23-26 "
Old	Turnip extract	"	30-34 "
"	Water	"	none in 96 hours

The points brought out in the above table are as follows:

1. The time required for infection by the old spores is always greater than that in the case of the corresponding infection by young spores: in the present experiment about twice as long in each case.

2. In the case of *Cereus*, both types of spore attack the petal almost as readily when sown in water as when sown in turnip extract. In the case of *Gloxinia*, the spores sown in water attack distinctly more slowly than the corresponding sowings in turnip extract. This is especially the case when the old spores are sown in water. Here no attack takes place even after four days, thus showing that the spores have been unable to obtain an appreciable amount of nutrient from the plant. When, on the other hand,

these old spores are sown in water on *Cereus* they attack readily enough, and for the reason that they obtain the nutrient necessary for their germination by passive exosmosis from the plant.

In a second experiment use was made of the fact that a certain concentration of carbon dioxide in the atmosphere inhibits the germination of *Botrytis* spores when sown in water, whereas the effect is relatively much less when the spores are sown in nutrient. Young spores of *Botrytis* were sown in water on petals of Rose and *Cereus*, in the one case in air, in the other in an atmosphere composed of 80 per cent. air and 20 per cent. carbon dioxide. In air attack readily took place in both cases, but in the carbon dioxide atmosphere only the *Cereus* was attacked. Even after three days the spores on Rose in the latter case were ungerminated. These results show that in *Cereus* the exosmosis of nutrient is sufficient to stimulate the germination of the spores in spite of the antagonistic effect of the carbon dioxide, whereas in the case of Rose the amount of exosmosis is insufficient for this purpose.

A third type of experiment was based on the fact that spores of *Botrytis* which germinate in pure water can be inhibited from doing so by sowing them sufficiently densely. This inhibiting effect is removed by the addition of a sufficiency of nutrient. Thus, while the effect of increasing the density of spore sowing is to diminish the amount of germination when the spores are sown in water, no such effect is obtained (at any rate within the same limits of spore concentration) when the spores are sown in nutrient.

In the case of *Cereus* petals which give high exosmosis figures, increase of spore concentration has no appreciable effect on the time of establishment of attack. In cases where the amount of exosmosis is much less, increase of concentration of spores leads to delay in the time of attack. Thus in an experiment with petals of Sweet-pea the following figures were obtained :

<i>Relative Density of spore sowing.</i>	<i>Number of inoculations.</i>	<i>Number of infections after 20 hours.</i>	<i>Percentage of infections.</i>
1, 10	57	49	86
1	53	30	57
10	41	1	2.5

These results are readily interpreted on the lines of the higher rate of exosmosis from *Cereus* and the smaller rate from Sweet-pea.

* The effect of increased density of spore sowing was not so clearly marked as was anticipated. Though only one of the forty-one inoculations with the densest spore suspension had taken in twenty hours, nevertheless they all showed infection ultimately. Total inhibition was expected on the ground that when a drop of the spore suspension was added to a drop of water which had lain on the petal for several days no germination took place. A more striking illustration of this discrepancy was met with in

experiments with Rose petals, where it was found that infection could be produced even when the spores were put on so thickly as to constitute a paste. Thus, while in the germination tests on glass it is readily possible to inhibit germination by increasing the concentration of spore sowing, the same is not possible in sowings on the petal itself.

On examining the dense suspension of spores which had begun to give infection of the petal it was seen that only a few spores had germinated, and these in the neighbourhood of the spots where attack was beginning. The apparent discrepancy is therefore probably explicable on the ground that in the case of the germination tests on glass the small quantity of nutrient is divided between a very large number of spores and produces no apparent effect as regards germination, whereas in the case of the infection drops on the petal itself the nutrient reaches the drop unilaterally, and probably therefore is completely taken up by the spores lying in contact with the plant surface. The latter would thus be able to germinate and produce infection while the great bulk of the spores present remain ungerminated and ineffective.

EXOSMOSIS FROM FOLIAGE LEAVES.

The examination of foliage leaves in the present connexion has not been carried out in the same detail as in the case of floral structures. The reason for this lies in the fact that difficulty was experienced in obtaining a suitable plant and a suitable fungus for investigation.

All the petals examined show the common feature that they are readily attacked by spores of *Botrytis* when the latter are placed on the petal in suspension in pure water. In such a case therefore it is possible to examine the phenomenon of exosmosis in relation to attack. Up to the present, however, no plant has come under observation the leaves of which can be parasitized by *Botrytis* in this manner. Even with the leaves of Broad Bean, which have been so much used in investigations of parasitism of the *Botrytis* type, no satisfactory infection is shown when the fungal spores are placed on the leaf in pure water, though it takes place readily when extraneous nutrient is supplied. In these circumstances one has to be contented with examining the negative side of the problem, i. e. the relation between the particular exosmosis features presenting themselves and the absence of attack.

Measurements have been made of the exosmosis from leaves of Broad Bean, French Bean, and *Tradescantia discolor*. In all these cases very complete wetting of the leaves took place and high figures were obtained in the conductivity determinations. Thus in one experiment where a comparison was made between Beans grown in the open at ordinary temperature and others forced in a greenhouse at a higher temperature, the following figures for conductivity were obtained. (The leaves were all

thoroughly rinsed in water beforehand to remove any foreign matter from the surface.)

Beans grown in open 13.4, 13.8, 13.8, 17.6

Beans forced in hothouse 31.9, 35.6, 44.3, 49.2

The exosmosis from the forced plants was considerably greater than that from those grown in the open, and in the case of both was greater than what is typically obtained from petals. Nevertheless these drops, when treated according to the method already described, had no appreciable effects in stimulating spore germination. They were found to be practically identical with distilled water in this respect.

The behaviour of the leaves in the present experiment as regards susceptibility to attack by *Botrytis* was found to be as follows:

Beans grown in the open—spores sown in nutrient—attack commencing in 22 hours.

Beans grown in the open—spores sown in water—no attack in seven days.

Beans grown in hot-house—spores sown in nutrient—attack commencing in 16 hours.

Beans grown in hot-house—spores sown in water—only doubtful attack in 7 days.

When the spores are supplied with extraneous nutrient, attack takes place readily in both cases, and it will be noticed that the time for the first signs of attack to appear is shorter for forced leaves than for leaves grown in the open. This feature is no doubt correlated with greater development of the cuticle in the latter case.

The slight amount of attack of the forced leaves by spores sown in water is of no interest, as it was observed that the leaves had markedly deteriorated during the long treatment. It can be truly stated that neither type of leaf shows any susceptibility to attack when the spores are sown in water. The non-susceptibility of these leaves is thus in complete agreement with the fact that the substances passing out have no nutrient value for *Botrytis* spores.

In the examination of some leaves an interesting result was met with, viz. that drops which had lain for some time on the surface of the leaf, and which were then found to possess a relatively high conductivity, actually produced less germination than did the distilled water controls. This result has been obtained in some cases with Bean leaves. The following table relating to an experiment with leaves of *Tradescantia discolor* illustrates this effect:

Conductivity of drop from leaf.	Effect on germination.
16.4	Very similar to water control.
26.5	Not so good as water control.
26.1	Similar to water control.
43.1	Distinctly less than water control.
50.2	Almost completely inhibited.

Here, apart from slight irregularities, the amount of germination runs more or less inversely to the conductivity. The leaves had not been treated to a preliminary washing, so that the source of the conducting material cannot be definitely stated. It may have come mainly from the interior of the leaf or may have been present to a large extent as some kind of incrustation on the surface.

Though the present experiment relates to a case where there is no question of parasitic attack (it was found that only the most meagre attack took place even when the spores were sown in nutrient), nevertheless it is interesting as showing that spores when placed in distilled water on the surface of a leaf come under the influence of substances which are able to exercise an important effect on their germination.

It will be noticed incidentally that the correlation which was shown to exist between the figures for conductivity and for germinative capacity does not hold as from plant to plant. A drop of a given conductivity obtained from Bean leaves has a much smaller effect in stimulating germination than one of the same conductivity obtained from *Cereus* petals. Thus, apart from quantitative differences, there are qualitative differences between the substances diffusing out in the two cases.

EFFECT OF THE PRESENCE OF SPORES IN THE DROP ON THE RATE OF EXOSMOSIS.

In an earlier paper of this series it was shown that the active principle obtained from *Botrytis* spores is unable to diffuse through the cuticle. Blackman and Welsford confirmed this result by showing that the first visible change in the cells of the host plant took place only after actual penetration of the cuticle had been effected. On the basis of these observations the conclusion was drawn that the fungus has no effect on the host until penetration of the cuticle has taken place. The presence or absence of spores in the drop would thus be immaterial as regards the rate of exosmosis into the drop so long as infection was in abeyance. This deduction has been strikingly confirmed by the use of the conductivity method.

The method of experimenting was as follows. Flowers of Sweet-pea proved to be most satisfactory for this purpose. They furnished very uniform conductivity figures and being symmetrical one side could be used as a control to the other. Drops of water were laid on the one side (e.g. the right wing and the right half of the standard), and drops of spore-containing water on the other side (left wing and left half of standard). Examination of the drops was begun an hour or so before infection began, the time required for this being ascertained by preliminary experiment. The conductivity of each drop was then measured at hourly intervals, the drops being replaced as far as possible on the part of the

petal from which they had been taken. Side by side with these readings the petals were closely examined for the first macroscopic appearance of infection, i.e. for the presence of small discoloured spots or, what was found to be a better test, for the presence of small translucent patches when the petals were viewed by transmitted light. In this way the progress of infection was studied side by side with the rate of exosmosis into the drops. The following table gives the results obtained in one particular experiment :

An * denotes that in the intervals so marked infection could be seen to have begun.

Conductivity at the following intervals from sowing :

		5 hrs.	6 hrs.	7 hrs.	8 hrs.	9 hrs.
<i>Spore-free drops.</i>	1.	2.17	3.81	5.73	6.64	7.99
	2.	2.30	4.16	5.29	6.29	6.98
	3.	2.38	4.21	5.58	6.64	7.54
	4.	2.44	4.16	5.29	6.05	6.84
	5.	2.48	4.21	5.39	6.26	6.89
	6.	2.48	4.31	5.41	6.31	7.18
	7.	2.48	4.66	6.29	6.58	7.67
	8.	2.55	3.87	5.22	6.03	6.89
	9.	2.82	4.84	6.45	7.51	8.55
	10.	2.82	5.04	6.84	7.34	8.05
	11.	2.89	4.58	5.92	6.92	7.64
	12.	2.99	4.93	6.56	7.61	8.94
	13.	3.21	5.08	6.21	7.21	8.02
	14.	3.27	4.60	5.77	6.86	7.58
<i>Spore-containing drops.</i>	1.	2.18	3.61	4.90	6.25	8.48
	2.	2.32	3.95	5.20	6.39	8.35
	3.	2.36	3.95	5.18	6.48	8.32
	4.	2.50	3.64	*6.78	19.35	41.41
	5.	2.69	4.45	6.23	*9.03	13.06
	6.	2.92	4.45	*5.72	6.64	8.08
	7.	3.06	4.73	*6.31	9.37	18.76
	8.	3.09	4.64	*6.34	8.45	12.57
	9.	3.16	4.49	5.80	6.86	8.18
	10.	3.28	4.82	6.16	7.04	7.99
	11.	3.83	5.90	*8.45	13.77	23.42
	12.	3.89	6.34	*11.10	19.54	31.57
	13.	3.99	5.90	*9.59	18.76	31.70
	14.	4.51	*7.89	17.66	33.75	—

At the end of five hours from sowing no attack was visible in any case on macroscopic examination. At this time the average conductivity of the fourteen drops which contained spores was 3.11, that of the controls 2.67. The initial conductivity of the spore suspension was 1.3, that of water 0.9. Making this correction it is seen that the difference in rates of exosmosis in the two cases is negligible.

Throughout the whole series it is seen that the rate of increase in conductivity is very similar in the spore-containing drops to that in the controls until such time as infection was seen to have occurred. When this happened the conductivity of the spore-containing drops in general rapidly rose to high values, the surface of the petal in contact with the drop becoming in the meanwhile entirely discoloured through the coalescence

*of a large number of discoloured spots. In some cases the first attack might be very localized, only one spot appearing for a considerable time, in which case the rise in conductivity in the drop subsequent to attack took place more gradually (No. 6 of spore-containing drops in the preceding table).

By the time infection can be detected macroscopically it has already progressed to some extent; the first stages of attack can only be demonstrated microscopically. This is readily done by taking a thin surface section of the petal and viewing it from above in the field of the microscope. Penetration, when it has taken place, is shown by the different appearance of the portion of the hypha within the tissue, the margins being more hazy than those of the hyphae external to the tissue, and also by the necessity of focusing at a lower level. Further, the internal portion of an invading hypha appears to follow a direction entirely independent of the original (external) hypha, and so the point of penetration is almost invariably marked by a sharp bend in the hypha when the latter is looked at from a direction at right angles to the leaf surface.

In experiments of the above type it was repeatedly proved that infection had already taken place in cases where there was no visible macroscopic discoloration and where there was no evidence whatever of any tendency towards an increased rate of exosmosis into the spore-containing drops as compared with the controls. Infection could thus always be demonstrated by ocular means before any effect on exosmosis, due to the presence of the fungus, appeared;¹ in other words, the rate of exosmosis into the spore-containing drops is the same as into the spore-free drops until such time as penetration has taken place in the case of the former. According to the view of 'action in advance of penetration' put forward by de Bary, the rapid exosmosis of nutrient substances from the host cells takes place antecedent to penetration, and is in fact responsible for the germination of the fungal spores and for their attack. It is clearly shown, however, by the preceding data that penetration has already taken place before the rapid exosmosis due to killing of the host cells has begun. In fact, it is only some time after infection that any divergence in the rates of exosmosis between the spore-free and the spore-containing drops takes place. This lag would be explained on the basis of the time required for the invading hyphae to kill the adjacent cells of the host and for the soluble contents of the killed cells to diffuse out into the infection drop.

These results are thus in complete agreement with the views put forward in the earlier papers of this series and in opposition to the view of de Bary on this subject.

¹ Indeed, in one very fortunate case, penetration of the cuticle was seen to have taken place while the host cell immediately underlying the point of penetration had not yet lost its colour and was still plasmolysable.

DISCUSSION OF RESULTS.

It would be premature, with the data at present available, to try to estimate the significance of the foregoing results in relation to the physiological processes of infection. The present paper deals mainly with floral organs, and thus touches only a small and unimportant part of the field of parasitism. As has been already stated, the difficulty met with in the treatment of foliage leaves lay in the fact that no satisfactory parasitism of the leaves investigated could be brought about by *Botrytis*. Thus the examination of foliage leaves according to the method of the present paper could be carried out only in its negative aspect. With a view to the further development of this problem an investigation of some fungus more suitable for this purpose than *Botrytis* is projected.

In all the cases examined it has been found that drops of water when laid on the surface of plant structures show an increase of conductivity, which increase has been found in a number of instances to be accompanied by an increased capacity for stimulating germination. The increase of conductivity, due to leaching of electrolytes from the plant, is in agreement with a large body of recent work dealing with the permeability of the living cell-membrane to electrolytes (and other simple solutes) and requires no further emphasis here. From the pathological point of view the essential point brought out is that water drops when laid on plant structures are altered in respect of their capacity to stimulate spore germination. Drops so treated show in some cases a very marked increase in this respect; in other cases the effect obtained is much less; and finally cases were met with in which the treatment actually reduced the amount of germination as compared with that taking place in the water controls. As it has been proved in earlier papers that the fungal spores exert no influence on the host previous to penetration of the cuticle, it is obvious that the preceding results which were obtained for drops of water laid on the plant are directly applicable to the infection drops themselves. The amount and quality of the substances diffusing into the infection drop must obviously exert an important influence on the behaviour of spores. Where the substances diffusing out from the plant have a marked stimulating effect on germination, the fungal spores when sown in water will germinate as in nutrient and will therefore be able to attack the plant with the vigour induced by good nutrition. Where the substances diffusing from the plant are such that they exert no appreciable influence one way or the other on the germination of the fungal spores, the course of affairs will be largely dependent on the inherent vigour of the spores. If these are young, a certain amount of germination will take place and attack may in some cases follow; if they are old (or in other ways attenuated),

germination will not take place and no attack will be possible. Lastly, where the substances diffusing from the plant are such as to inhibit the spore germination, it is obvious that no attack can take place in the circumstances.

It is well known that in some cases immunity is in no way related to considerations such as those outlined above. Thus in some of the Rusts it has been shown that both immune and susceptible varieties of the host are actually penetrated by the fungus. The differential effect appears only after penetration has taken place, inasmuch as further growth of the fungus is inhibited in the case of the immune variety, whereas it continues in the other. Here, therefore, the immunity or susceptibility of the host is not due to conditions prevailing in the infection drop, but to different and more complex relationships between host and parasite which come into play only after penetration has taken place. Nevertheless, there are cases where attack is determined by the conditions prevailing in the infection drop. The parasitism of the Bean leaf by *Botrytis* is a case in point. Here the incidence of attack is dependent on whether sufficient nutrient is present in the infection drop to enable the fungus to germinate and penetrate the cuticular layer. If nutrient is present, attack takes place with readiness; if no nutrient is present, the attack fails. It is to parasitism of this type that the present results apply, and in which the methods of investigation outlined above may prove of value in determining just why attack fails in some cases and why it succeeds in others.

SUMMARY.

1. Drops of distilled water which have lain on the surface of leaves and petals of a number of plants show increased conductivity as compared with the original distilled water or with water which has lain for an equal time on glass slides.
2. This increase in conductivity is accompanied in many cases (e.g. floral leaves) by a greatly increased capacity of the drops to bring about germination of *Botrytis* spores as compared with that of the original water. In some plants drops so treated, though showing a comparatively high degree of conductivity, have no greater effect on germination than drops of pure water. In some cases, even, the treated drops actually produce total inhibition of the fungal spores.
3. In the case where increased germination effects are observed the amount of germination runs parallel with the conductivity.
4. The ease or difficulty with which wetting of the plant surface takes place is an important factor in determining the magnitude of the effects produced.

5. Indirect proof of the exosmosis of nutrient can be obtained by a study of the incubation times of infection in different cases.

6. The rate of exosmosis into drops containing *Botrytis* spores is identical with that into spore-free drops, up to and for some time after penetration by *Botrytis* has taken place. The rate of exosmosis then increases with great rapidity in the case of the infection drops.

The writer wishes to express his indebtedness to Mr. S. G. Crater, of Rocky Mount, North Carolina, for his help in the experiments dealing with the effect of age on the permeability of petals, as described on p. 104, and also for repeating and verifying a number of other results.

The Flowering Curve of the Egyptian Cotton-plant.

BY

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With three Tables and seven Figures in the Text.

THE introduction of the more precise methods of the plant physiologist in the analysis of agricultural experiments has been particularly

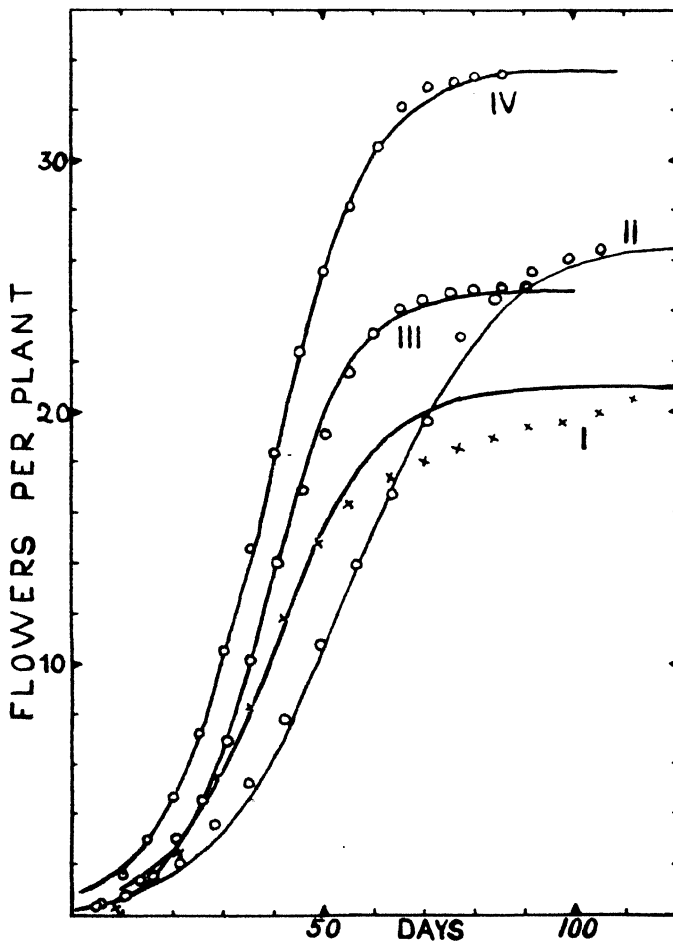


FIG. 1. Flowering curves of Cotton. I, data of W. L. Balls, Giza, 1909. II, ditto, 1911. III, Bahtim, 1917. Three years' rotation; farm-yard manure plots. IV, Bahtim, 1920. Pilon, varieties experiment.

successful in the case of the Egyptian cotton crop. Flower counts, boll counts, and growth data, all first inaugurated by W. L. Balls, are now extensively used in Egypt. Since the season of 1917 a large number of flowering curves have been obtained at the Bahtim Experimental Station relating to cotton under many different conditions of experiment. Although the intensity curve of flowering (flowers per plant per day) is of greater interest to the physiologist, yet to the agriculturist a summation curve represents much more satisfactorily the actual condition of the plant at any given moment, and these curves have been used at Bahtim since the present

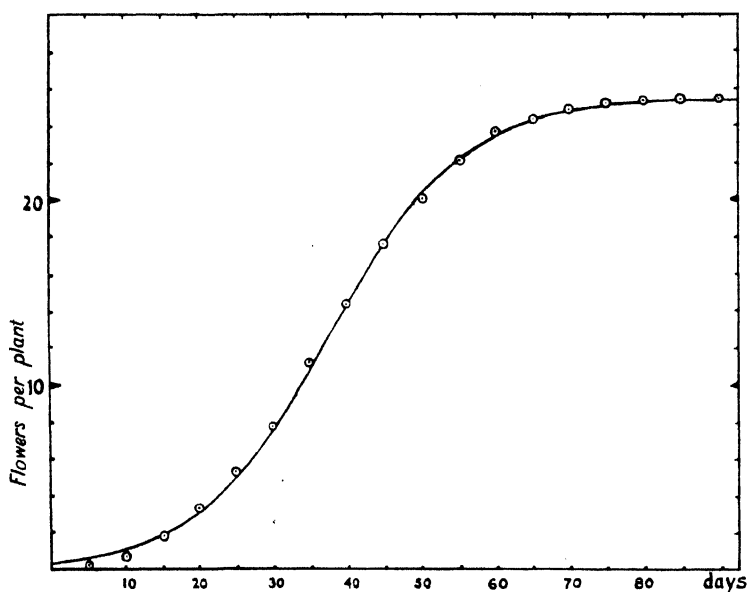


FIG. 2. Flowering curve of Cotton, Bahtim, 1920. Sakellaridis, plants topped and water restricted in August.

series of observations was started. One striking feature of the summation curve (total flowers per plant up to the date given) was the fact that the irregularities were more or less smoothed out and a characteristic S-shaped curve was always obtained. Other data published by W. L. Balls (1912) and B. G. C. Bolland (1917) gave similar summation curves.

It will be seen from the analysis of some of the typical records that this summation curve can be expressed with considerable accuracy by the following equations:

$$\frac{dx}{dt} = kx(a-x) \quad \text{or} \quad \log_e \frac{ax}{a-x} = kat \quad \text{or} \quad \log \frac{x}{a-x} = K(t-t_1),$$

where a is the final number of flowers eventually obtained, x is the number of flowers up to time t . k and K are constants where

$$K = ak \log e = 0.4343 ak,$$

and t_1 is the time when $x = \frac{a}{2}$.

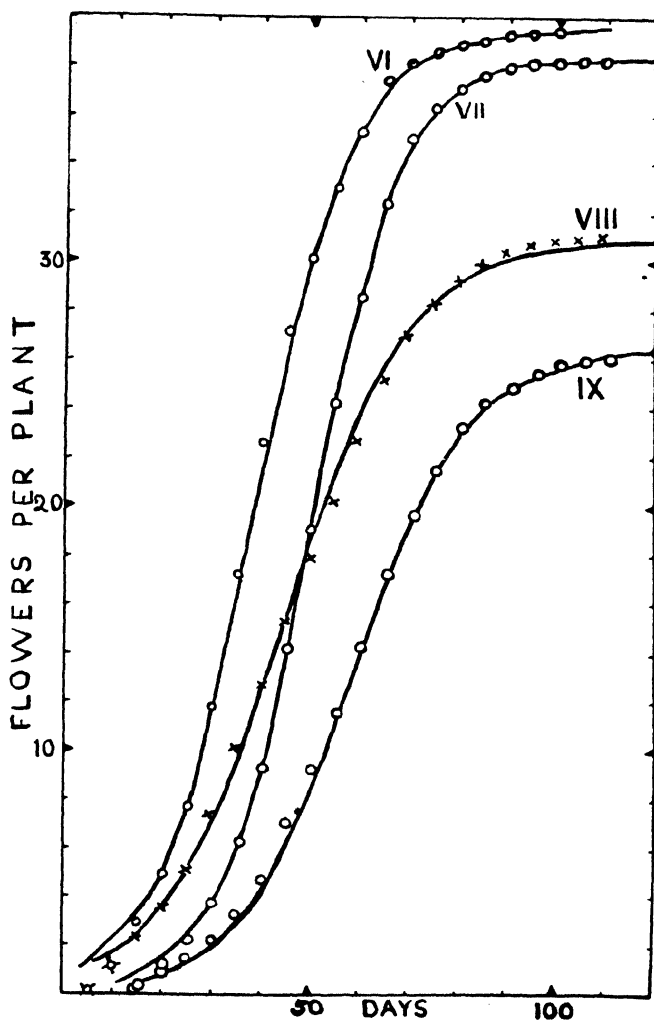


FIG. 3. Flowering curves of Cotton from dates of sowing experiment, Bahtim, 1920. VI, Pilion sown March 1. VII, Pilion sown April 1. VIII, Sakellaridis sown March 1. IX, Sakellaridis sown April 1.

The origin of the time scale can be taken at any point, but usually the date of the first flower is taken and the time counted in days from this point. t_1 fixes the position of the curve as a whole along the time scale.

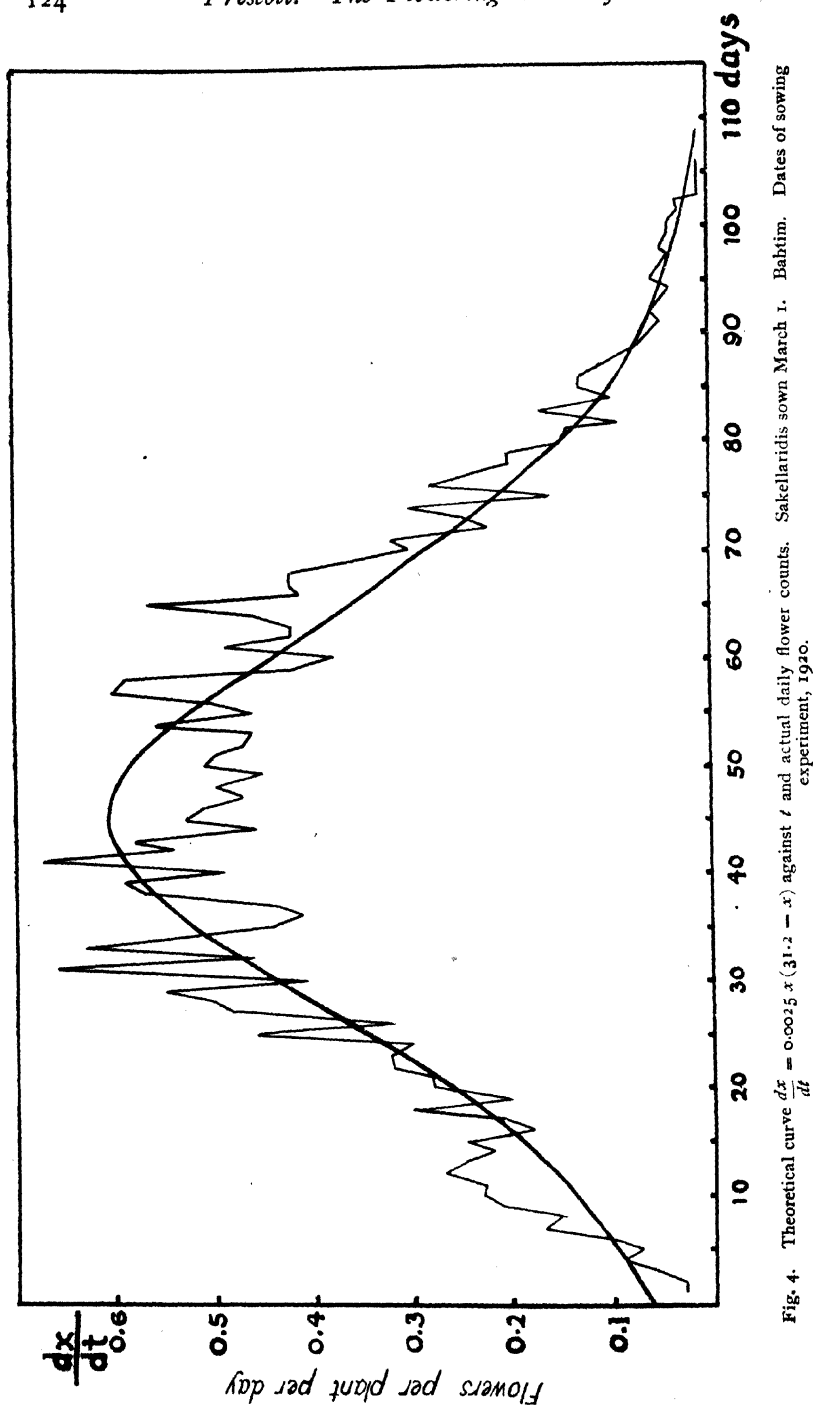


Fig. 4. Theoretical curve $\frac{dx}{dt} = 0.0025 \times (31.2 - x)$ against t and actual daily flower counts, Sakellaridis sown March 1. Baktim. Dates of sowing experiment, 1920.

The equation is that of an autocatalytic chemical reaction and was originally formulated by W. Ostwald, who pointed out the similarity between growth processes and autocatalysis. T. B. Robertson (1908-1915) has elaborated the idea still further and prepared a series of tables for use in connexion with this equation. H. S. Reed (1920), working in California, has shown that the equation can be applied in many cases, and gives data for pear-trees and *Helianthus* so as to show the applicability to earlier published data on growth.

The flowering curve of the cotton-plant is probably particularly suited for analysis along these lines. W. L. Balls (1915) has already shown that

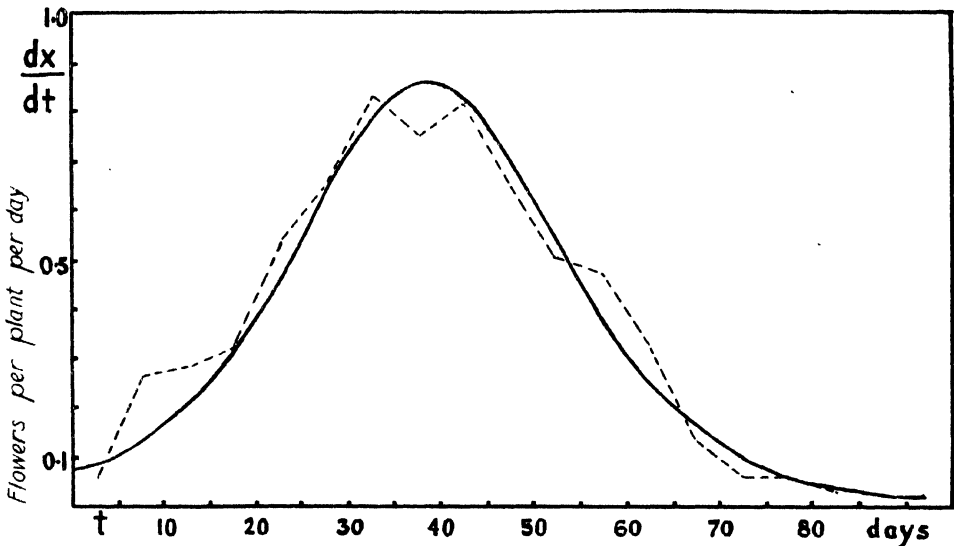


FIG. 5. Flowering curves (flowers per plant per day) of Cotton-plants. Pilon. Variety test. Bahtim, 1920. The broken line indicates the familiar five-day mean flowering curve. (See also Fig. 6.)

the flowering curve reproduces in its earlier stages the growth curves of the preceding month, and there is no doubt that the curve as a whole gives a very fair representation of the growth of the plant. The simplicity with which flowering records can be obtained, the unitary character of the records independent of measurements either of length or of weight, and the large number of plants which it is possible to observe, make it possible to present data of reasonable accuracy and to smooth out irregularities due to individual plant variations. In most of the cases quoted below counts have been made on four hundred plants.

The method employed in obtaining the curves is illustrated from the data given in Table I, following the methods usually adopted by the physical chemist. For a number of values of x and t the corresponding

value of K has been calculated, and it will be seen that K is practically constant over the whole range of the observed data. From the average value of K the actual theoretical curve can be calculated, and this has been done in a number of cases which are given in the figures. The original data from which the values of the constants have been calculated are given in Table II and the constants themselves in Table III.

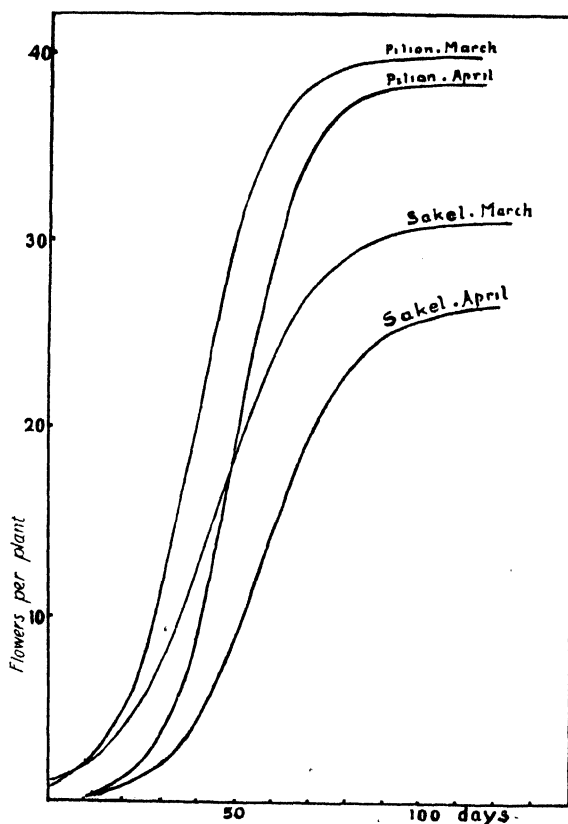


FIG. 6. Flowering curves of the dates of sowing experiment, Bahtim, 1920. Sakellaridis and Pilon are well-known commercial varieties.

In Figs. 4 and 5 are given the $\frac{dx}{dt}$ calculated curves together with the familiar flowers per plant per day; in one case (Fig. 4) the actual daily counts are given, while in Fig. 5 the more usual five-day mean curve is given. It is only possible to smooth out these intensity curves after the summation curve has first been constructed from the data and then analysed along the lines given in this paper.

In Fig. 1 (1), representing the flowering curve given by W. L. Balls for

Giza, 1909, it is interesting to note that the curve has been cut off rather sharply after July 30. It is significant to note that according to W. L. Balls the yield at Giza in 1909 was cut down very early by the rising water table due to an exceptionally early and high Nile flood.

Very few, if any, of the Bahtim curves so far examined show this feature, and there is reason to believe from other evidence that the water table plays

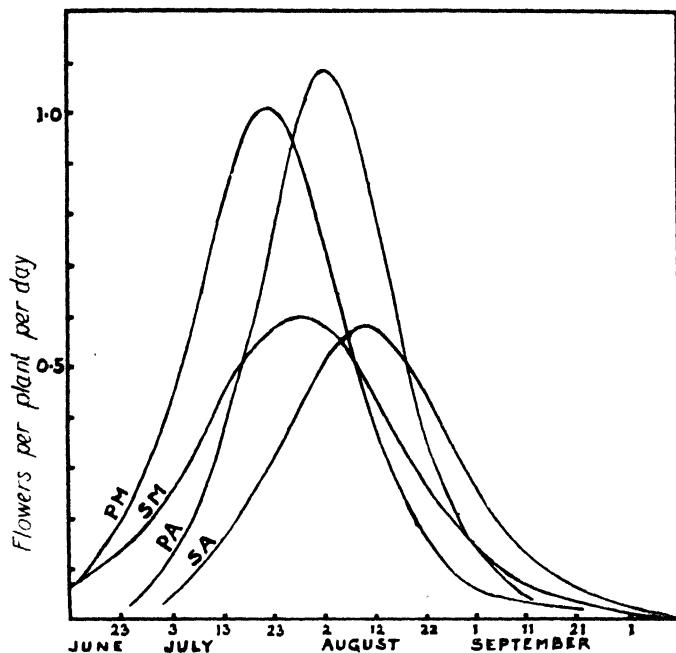


FIG. 7. Smoothed flowering curves (flowers per plant per day) of the Bahtim dates of sowing experiment, 1920. PM, Pilion sown March 1. PA, Pilion sown April 1. SM, Sakellaridis sown March 1. SA, Sakellaridis sown April 1.

a relatively unimportant part in the Bahtim experiments. It ought to be possible by this method of analysis of the summation curves to indicate reasonably sharply the influence of any specially marked disturbing factor on the yield of the plant.

The possibility of the application of a series of these curves to an agricultural experiment is illustrated in Figs. 6 and 7, which give the flowering curves calculated from the data of the dates of sowing experiment at Bahtim in 1920.

TABLE I. Flowering Records of Cotton-plants. *Baktim Experiments*
Dates of Sowing, 1920.

Pilion sown April 1. See Figs. 3 (VII), 7.

$$a = 38.5. \quad t_1 = 50.$$

Observed values.		K (calculated).	Calculated values from which curves are drawn.		
Flowers per plant.	Days.		x	t	$\frac{dx}{dt}$
0.0	5		1	17.9	0.110
0.0	10		2	24.3	0.214
0.3	15	(0.060)	4	30.9	0.404
1.2	20	0.050	5	33.1	0.491
2.3	25	0.049	8	38.1	0.715
3.7	30	0.049	10	40.7	0.835
6.2	35	0.048	12	43.0	0.932
9.4	40	0.049	15	46.0	1.033
14.2	45	0.047	18	48.8	1.082
19.2	50	0.047	20	50.7	1.084
24.4	55	0.048	22	52.3	1.063
28.7	60	0.047	25	55.5	0.989
32.3	65	0.048	28	58.7	0.861
35.2	70	0.051	30	61.2	0.747
36.5	75	0.050	32	64.1	0.609
37.3	80	0.049	35	70.4	0.359
37.8	85	0.049	36	73.6	0.264
38.2	90	0.052	37	78.4	0.163
38.3	95	0.051	38	88.6	0.056
38.3	100	0.046	38.3	96.6	0.022
38.4	105				
38.4	110				

Average value of $K = 0.049$

$$k = \frac{0.049}{0.4343 \times 38.5} = 0.0029$$

TABLE II. *Flowering of cotton-plants. Flowers per plant up to date given. Bahim experiments.*

Time in days from commencement of flowering.	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100	105	110
1917.	0.4	0.8	1.6	3.1	4.6	6.9	10.2	14.1	17.0	19.2	21.7	23.3	24.2	24.5	24.8	25.0	25.1	25.1				
1920.	1.	0.2	0.7	1.8	3.3	5.3	7.3	11.3	14.4	17.6	20.1	22.1	23.7	24.4	24.9	25.2	25.4	25.6	25.7			
1.	0.3	1.6	3.0	4.6	7.3	10.5	14.7	18.4	22.5	25.8	28.3	30.7	32.3	33.0	33.3	33.6	33.7					
2.	0.2	1.3	3.0	5.0	7.8	11.8	17.3	22.7	27.4	30.4	33.2	35.6	37.6	38.4	38.8	39.1	39.3	39.5	39.6	39.7	39.7	39.8
3.	0.0	0.0	0.3	1.2	2.3	3.7	6.2	9.4	14.2	19.2	24.4	28.7	32.3	35.2	36.5	37.3	37.8	38.2	38.3	38.3	38.4	38.4
4.	0.3	1.1	2.3	3.5	5.2	7.5	10.2	12.7	15.4	17.9	20.3	22.8	25.2	27.1	28.3	29.4	30.0	30.5	30.8	31.0	31.1	31.1
5.	0.0	0.0	0.2	0.8	1.4	2.2	3.3	4.8	7.0	9.3	11.7	14.5	17.1	19.8	21.7	23.5	24.5	25.2	25.8	26.1	26.3	26.4
6.																						

Flowers per plant calculated from data of W. L. Balls, 'The Cotton-plant in Egypt', p. 65 (1912).

Time in days from beginning of flowering.	7	14	21	28	35	42	49	56	63	70	77	84	91	98	105	112
Giza, 1909.	0.3	0.9	2.4	5.3	8.2	11.9	14.8	16.3	17.3	18.0	18.5	19.0	19.4	19.6	20.0	20.6
1911.	0.4	1.3	2.0	3.5	5.2	7.8	10.7	13.8	16.7	19.6	23.0	24.5	25.5	26.0	26.5	

TABLE III. *Values of constants used in plotting the flowering curves in Figs. 1-7.*

Variety and treatment of cotton-plants.	a	K	k	t ₁	Date of origin, t = 0.
Giza, 1909 (W. L. Balls)					
Giza, 1911 (W. L. Balls)					
Bahim, 1917. Three years' rotation Sakellariadis					
Farm-yard manure plots:					
1. Sakellariadis. Plants topped and watering restricted in August	25.7	0.047	—	38.0	June 10
2. Pilon. Variety test	34.0	0.044	0.0030	38.5	June 13
3. Pilon sown March 1. Dates of sowing experiment	40.0	0.044	0.0035	39.0	June 13
4. Pilon sown April 1. Dates of sowing experiment	38.5	0.049	0.0029	50.0	June 13
5. Sakellariadis sown March 1. Dates of sowing experiment	31.2	0.034	0.0045	45.0	June 13
6. Sakellariadis sown April 1. Dates of sowing experiment	26.6	0.038	0.0033	58.0	June 13

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H. S. REED (1920): Journal of General Physiology, ii. 545-61. The Nature of the Growth Rate.

NOTE.

ABNORMAL FLOWERS IN ERANTHIS.—The genus *Eranthis* consists of about ten described species inhabiting the Mediterranean Region and Central and Eastern Asia. Morphologically it is very closely related to the genus *Helleborus*, and, as Dr. A. H. Church has pointed out ('Floral Mechanism,' i, p. 14, note 2), the generally adopted taxonomic division between the two genera is perhaps not the most natural one which could be adopted. The characters usually relied upon for separating them are: 1, the membranous deciduous sepals of *Eranthis* contrasting with the herbaceous persistent ones of *Helleborus*; 2, leaves palmate in *Eranthis* and generally pedate in *Helleborus*; 3, ovules with two integuments in *Eranthis* and with one in *Helleborus*.

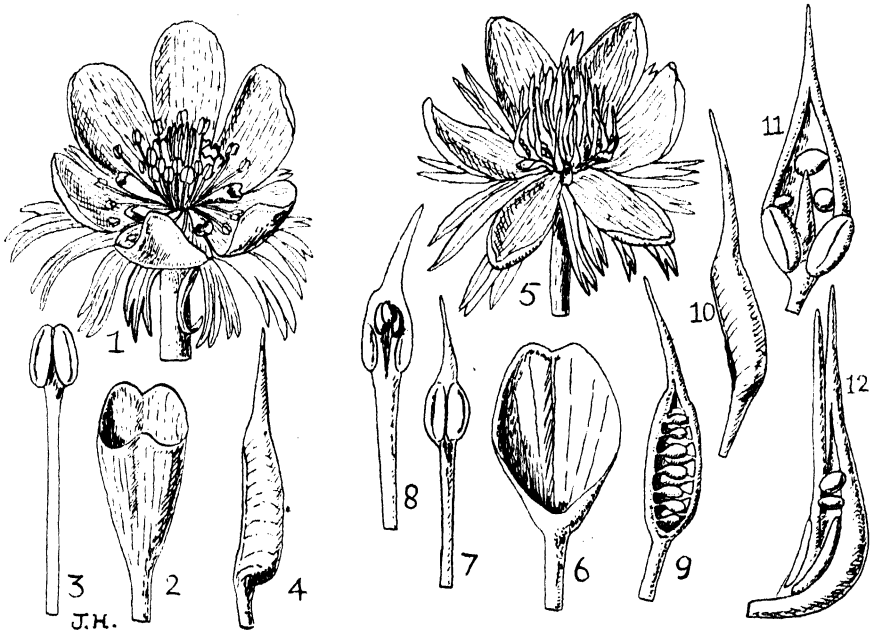
E. cilicica, Schott et Kotschy, is a species which is geographically limited to Asia Minor, Syria, and Armenia. It is very closely allied to *E. hiemalis*, Salisb., but differs in having the involucre segments divided into more numerous and narrower lobes, ovate not oblong anthers, and erect not falcate carpels. Huth ('Engl. Jahrb.,' xvi, p. 297, 1892-3) reduces the species to a variety of *E. hiemalis*, Salisb., and this is probably the more reasonable view. Huth records the plant also from Mt. Delphi (Dirphys), Euboea, but no confirmation has been found of this record, and the genus is not referred to in Halácsy, 'Conspectus Florae Graecae'.

E. cilicica is regularly grown in the Alpine House at Kew, and in February of this year three pots were in full bloom. While examining the individual flowers with a pocket-lens the writer observed that all those of one plant showed some anomalies which are the subject of the present note. The flowers of all the other plants appeared perfectly normal in all respects.

The normal flower (Fig. 1) consists of six bright yellow sepals, about six, deep orange, tubular honey-glands or petals (Fig. 2), numerous stamens, and numerous multiovulate carpels. Immediately below the flower occurs a whorl of three green bracts which are divided more or less deeply into narrow segments. Each stamen (Fig. 3) has a slender filament averaging 6 mm. in length and an anther 2 mm. in length with two well-formed lobes, each of which is rounded above and below. Occasionally an outer stamen shows a smaller anther with the lobes narrower. A normal carpel (Fig. 4) is 8 mm. in length, and on the adaxial side it passes straight down into a short stalk, but on the abaxial side it bulges considerably and is then constricted suddenly before passing into the stalk. A gradually tapering style, a little shorter than the body of the carpel, surmounts the whole.

In an abnormal flower (Fig. 5) the yellow sepals were somewhat narrower than normally. The honey-glands were present to the number of five or six, and, while some were of the typical shape, most had a better developed slender stalk and showed an expanded blade, with a corresponding reduction in the tubular portion (Fig. 6).

Intermediate stages between the honey-glands and the stamens were not found, nor were such intermediate stages between sepals and honey-glands seen as are described and figured by Masters, 'Vegetable Teratology', p. 23, Fig. 9. In the abnormal flowers no completely normal stamens were observed, but interesting transitions between stamens and carpels were noted. Of these the most interesting are figured. Many of the stamens (as in Fig. 7) had each two well-formed lobes, but above these projected an elongation of the connective. A specially interesting condition is seen in Fig. 8, where an organ is depicted with both pollen-producing tissue and several ovules. Pollen grains, some of which appeared fully normal in shape and size, were obtained from one of these stamen-carpels. The pollen-tissue indicated the bilobed



condition of a perfect anther, while the ovules, always found above it, were exposed to view, but arose from the sides of a hood-like hollow. The organ terminated in what must be regarded as the equivalent of a style. Several of these stamen-carpels showed what seem to be both elongated connective and style. Thus in Fig. 11 two obvious but separated anther-lobes are seen, and from between them an elongated connective arises. The upper part of the organ is an open carpel, with several ovules, and terminates in a well-marked style. Fig. 12 shows a very similar condition, seen from the side, but the connective is very much elongated almost to an equal height with the style. It is to be noted that normally the anthers are introrse, and in the stamen-carpels the introrse pollen-tissue and ovules were both borne on the adaxial face of the organ. In other words, the Figs. 3, 7, 8, and 9 have the inner (adaxial) face showing. A stage which can only be described as a carpel split down the adaxial (ventral) suture is seen in Fig. 9. Many similar examples with the slit

more or less complete were examined. The ovules were generally arranged in two rows, one on each margin of the open carpel, and were smaller than ovules from a closed carpel of a normal flower. The ovules in a closed carpel occur in a single row on the adaxial suture. Fig. 10 indicates a carpel which is nearly normal, except that the constriction into the stalk is less abrupt and a slight split appears on the adaxial side.

Bonnier, in 'Bull. Soc. Bot. France', vol. xxvi, p. 139 (1879), describes some abnormal intermediate states between stamens and carpels in *Helleborus foetidus*. Unfortunately no figures are given, but from the description some at any rate of the intermediate conditions appear to agree very closely with some of those described here for *Eranthis* (Figs. 7-10). Bonnier does not, however, mention a condition of the 'stamen-carpel' where a prolonged connective and a well-developed style are both present. Since, as stated above, the two genera *Eranthis* and *Helleborus* are very closely related, it is of interest to find similar abnormalities recorded for species of both genera.

Many instances are noted by Masters ('Vegetable Teratology,' pp. 303-10), and some figured, of 'pistillody of the stamens'. Worsdell, too ('Principles of Plant Teratology,' vol. ii, pp. 182-93), mentions many cases of 'carpellody of the stamens', considering that this phenomenon reveals to us 'the fact that stamen and carpel are very closely allied organs, and the facility with which the one may change to the other, doubtless due to the fact that both are derived from a common ancestor, the asexual sporophyll, which exists to-day in some of the more primitive types of plants, such as ferns, horse-tails, and some lycopods'. None of the cases mentioned by Masters or Worsdell appears to be exactly equivalent to that described above for *Eranthis*.

My thanks are due to the Director of Kew for permission to publish this note, and to Mr. J. Hutchinson, F.L.S., for help with the figures.

W. B. TURRILL, M.Sc.

ROYAL BOTANIC GARDENS, KEW.

March 21, 1921.

The Leaf-skin Theory of the Stem: A Consideration of certain Anatomico-physiological Relations in the Spermatophyte Shoot.¹

BY

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With thirty-four Figures in the Text.

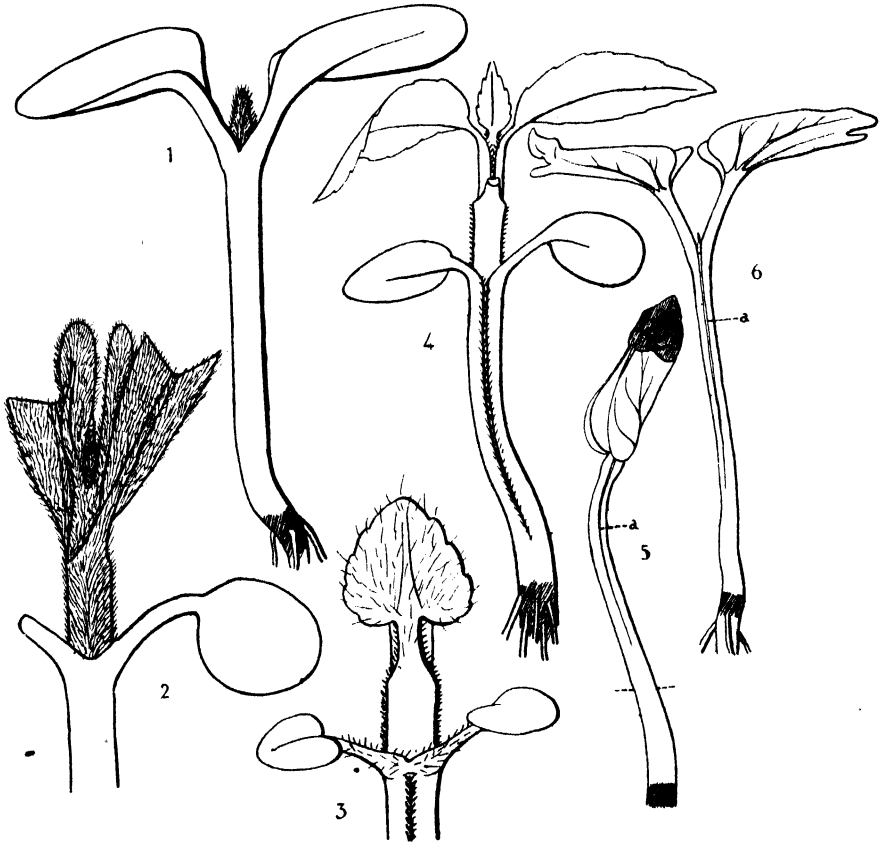
I. INTRODUCTION.²

(i) *THE original problem and the wider question which arose out of it.* In the course of investigations upon the inheritance of hairiness in *Matthiola incana*, necessitating examination of the young plant from the moment at which the plumule becomes visible, one cannot but be struck with the remarkably sharp line of demarcation, occurring at the cotyledon node, between the completely glabrous hypocotyl and the felt-covered plumule (see Fig. 1). As the shoot elongates it becomes apparent that the epicotyl axis is thickly covered with hairs as well as the foliage leaves, while the now fully developed cotyledons and hypocotyl remain glabrous (see Fig. 2). Confronted year after year with this sharp contrast one was inevitably led to speculate as to its cause. A study of many Spermatophyte seedlings has brought to light certain anatomical and physiological relations which appear not only to furnish a clue to the different character of the axis above and below the cotyledon node, so conspicuous in many species besides the Stock, but to throw light on the nature of the shoot axis in general.

(ii) *The nature of the evidence.* The observations from which the conclusions set forth below are derived are concerned almost wholly with the surface anatomy of the plant. They deal with relations some of which have been recognized and find mention in detailed systematic descriptions, although no particular significance seems to have been attached to them. Others, on the contrary, do not appear to have been noticed. Regarded by themselves and apart from all other considerations the anatomical features

¹ A brief preliminary communication on this subject was made at the Meeting of the British Association for the Advancement of Science in 1921. The present account covers a much wider field of observation, and the application of the leaf-skin conception is considerably extended.

here described will seem, no doubt, trivial in character, but viewed in the light of their relation to the plan of construction as a whole they assume a new importance and call for some explanation capable of general application. The sought-for solution, simple and obvious when once the facts have been grasped, is to be found in a definite (? universal) downward extension of the leaf area below the node level.



FIGS. 1-6. 1, *Matthiola incana*, seedling showing glabrous cotyledons and hypocotyl and hairy plumule. 2, older stage (lower leaf-tips removed). 3, *Veronica hederaefolia*, seedling; hypocotyl with a line of hairs along the potential edges of the cotyledon extensions. 4, *Lopezia coronata*, seedling plant; hypocotyl and internodes showing the same feature. 5, *Ipomoea sanguinea*, young seedling; a, the furrow formed between the edges of the cotyledon extensions. 6, older stage of the same.

2. GENERAL STATEMENT OF THE FACTS AND CONSIDERATION OF THEIR SIGNIFICANCE.

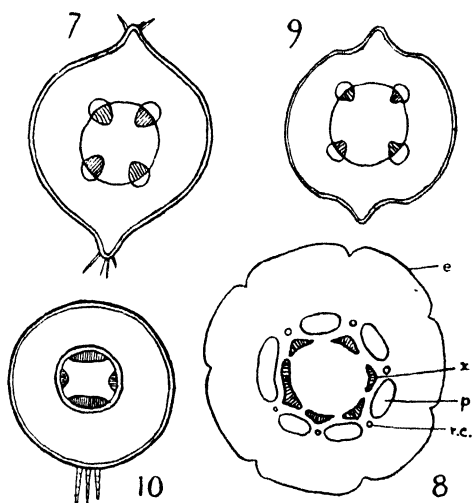
To return for a moment to the case of the Stock. It will be admitted that the absence of hairs from the cotyledons of a hairy-leaved form cannot be supposed to be entirely the result of an intra-seminal environment, since in some types the surface of the cotyledons is seen to be covered with hairs immediately they become free of the seed-coat. We need, therefore, to

look for some other explanation. Now it is to be observed that in some species the margins of the cotyledons become confluent at the level of insertion, completely enveloping the axis and forming a sort of cup or socket out of which the plumule emerges. This condition was found to exist in a very large number of the Dicotyledons examined and in several of the Gymnosperms. Where the cotyledons are of considerable thickness, as e.g. in Gorse (*Ulex europaea*) and Stock (*Matthiola incana*), the socket rim may be quite substantial (see Fig. 1); where they are less solid in structure it is correspondingly thinner. In the former case the hypocotyl has the same appearance all

round and is usually featureless, but in certain species coming under the latter head a distinct ridge or welt may be traced down either side from the point of coalescence of the cotyledons towards the root, as in *Rivina humilis*,¹ Plum. (Phytolaccaceae) (see Fig. 9). Or in place of a ridge there may be a well-defined line of hairs such as is seen under certain conditions in *Veronica hederacifolia* and *Lopezia coronata* (see Figs. 3 and 4), or, again, the two characters may coexist.

Thus in *Ruellia amoena* the ridge is ordinarily surmounted by a conspicuous line of characteristically shaped hairs, the rest of the surface being glabrous (see Fig. 7). When the seedling is growing under conditions which are still more favourable to hair

formation the hairiness may, however, become general, and in the same way *Rivina*, in which the ridge is more often glabrous, will sometimes develop a hair line under altered conditions. In the Polygonaceae the coalescence is emphasized by the production of a tubular sheath extending upwards for a short distance above the node level. In



FIGS. 7-10. 7, *Ruellia amoena*, hypocotyl in transverse section; the potential edges of the cotyledon extensions are thrown up into a ridge surmounted by hairs. 8, *Pinus maritima*, hypocotyl (after Chauveaud). e., epidermis; x., xylem; p., phloem; r.c., resin canal. 9, *Rivina humilis*, hypocotyl showing the same feature as *Ruellia* but without the hairs; the shallow groove on either side of the ridge results from the angled form of the cotyledon petioles. 10, *Stellaria media*, internode in transverse section.

¹ The presence in *Rivina* of a shallow furrow on either side of the ridge, indicated in the transverse section but more clearly seen in a surface view of the seedling, might suggest at first sight that this type should be classed with *Eucalyptus* and *Ipomoea* (see later). But the furrows in this plant have a different origin. A close examination shows that they are not intercotyledonary, but arise through a foreshadowing in the cotyledon stalk at its insertion of that four-angled shape which is assumed by the petiole of the leaves.

certain Gymnosperms several alternate ridges and furrows are visible in the hypocotyl, corresponding in number to the cotyledons, as e.g. in *Pinus maritima* (see Fig. 8) and *Picea orientalis*, Carr. We may place in another and probably much smaller category those species in which the width of the cotyledon insertion just falls short of half the circumference of the axis, so that the hypocotyl is not completely embraced. The result is a *double* contour line on each side extending down from the adjacent cotyledon edges and bounding a narrow longitudinal interspace which is seen as a slight furrow. This condition is well illustrated in species of *Ipomoea* (*I. coccinea*, *I. sanguinea* (see Figs. 5 and 6)) and *Eucalyptus* (*E. alpina*, *E. diversicolor*, *E. gomphocephala*), and can be observed as soon as germination has taken place. The contour lines in the above-mentioned *Ipomoea* forms, which contain a considerable amount of anthocyanin, may often be still further accentuated by a sharp colour contrast. The pigment is here confined to the first sub-epidermal layer, and in seedlings grown in damp shade it may colour the whole exposed region of the hypocotyl except the two furrows, which, together with the plumule with which they are continuous, appear of a beautiful translucent green when held to the light. Furthermore, the epidermal papillae which develop on the rest of the hypocotyl, giving it a pile-like surface, are absent from the furrows. At a later stage or under other conditions of illumination and moisture, anthocyanin may overspread these intervening strips, obliterating the colour boundaries but leaving the structural contour lines still plainly visible extending down the greater part of the hypocotyl, which, when fully grown, may measure from $1\frac{1}{2}$ to 2 inches in length. These shallow furrows, which are separated from the vascular ring by several layers of cortex, cannot be causally connected with any internal tissue distribution. Rather we are led by the facts adduced to regard ridge, furrow, hair line, and colour boundary as marking the limits in the superficial tissue of areas which are composed of downward extensions of the cotyledons between which, when the contours are double, appear similar prolongations of the next leaf-pair. In other words, this superficial tissue, i.e. the epidermis and one layer at least of the hypoderm (and possibly deeper layers as well), must be considered, so long as it is desirable and practicable to maintain this distinction, as *foliar* and not *axial* in nature. We can then look upon *Ipomoea*, *Lopezia*, and *Ulex* as representing gradations in a series of stages passing from a condition in which the cotyledon-extension edges are *actual*,¹ defined by anatomical and in certain circumstances by colour boundaries in addition: through that in which there is fusion of the contiguous margins, but a fusion of so superficial a character that the lines of junction exhibit features which we associate with *potential*

¹ In the sense that they are separate from each other although the whole extension is completely confluent with the axis.

edges: to one in which the union is sufficiently deep or so exactly adjusted that no anatomical demarcation is traceable. But though in this last case the boundaries are uncharted and signposts are wanting, we may safely presume that the development of the cotyledons follows the same course as in the two other types. When the potential edges are not only fused but become thrown out into ridges the explanation may be that growth in circumference in the cotyledons is more rapid than in the axial core, and that accommodation takes place in this way, while the condition in which the edges become actual may arise through the initial increase being greater in the core than in the cotyledons.

Now it is a familiar fact that anthocyanin production and hair formation are both characters which vary considerably in accord with environmental conditions. We may find, for example, that an *Ipomoea* grown exposed to full sunlight in dry soil has the entire hypocotyl surface uniformly coloured; or again, that some *Veronica* individuals will show a completely glabrous hypocotyl, while others have hairs distributed over the whole surface. *But the important point which concerns us here is not that under certain conditions hairs and anthocyanin can overspread the entire area, or on the other hand that their formation can be entirely suppressed, but that, under the appropriate conditions, the disposition of hairs, colour, or other anatomical features always follows a certain definite arrangement in relation to the morphological ground-plan.*

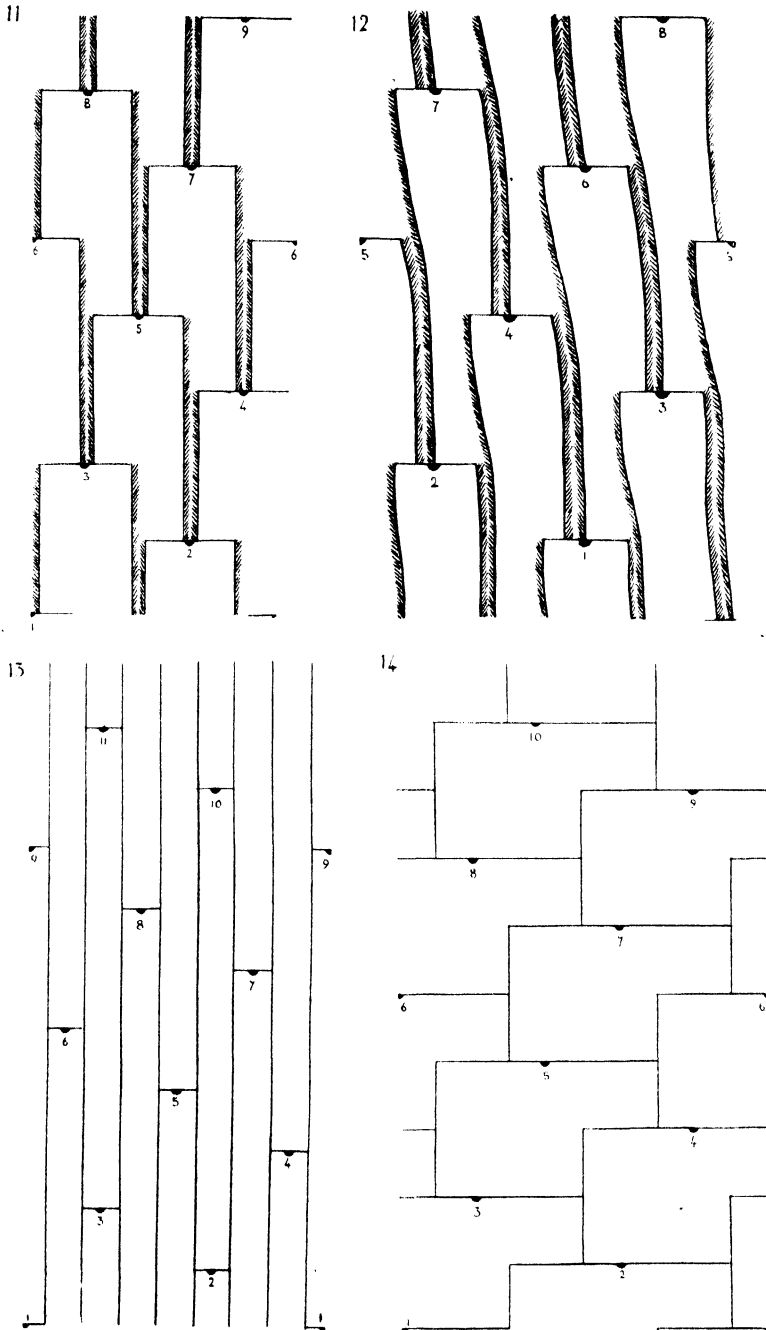
But if this view of the composite nature of the hypocotyl is well founded, are we to conceive of the epicotyl and the shoot thereafter produced from it as likewise consisting of a foliar skin enclosing an axial core? A careful examination of the stem surface shows that precisely similar and equally plain evidence is obtainable from the later developed as from the seedling shoot, the foliage-leaf extension being clearly outlined in many species by a furrow or ridge, or by a hair or colour line. And further, that where these topographical features are present they stand in a direct relation to (1) the general orientation of the individual, (2) the mode of phyllotaxis, (3) the proportion of the circumference of the axis occupied by the leaf-insertion. For example, if, in a species exhibiting one or other of these features, the leaf arrangement is decussate with a leaf-insertion width of half the stem circumference, then the congenitally fused but potential edges of the foliar extensions will show two longitudinal boundary or fusion lines in each internode, extending from the points where the free portions of each leaf-pair come into contact down to the mid-axils of the leaf-pair below, as e.g. in *Hypericum calycinum* and *Epilobium parviflorum* (see Fig. 21). If the insertion width is less than a half but more than a quarter of the circumference, there will obviously be, not two, but four such contour lines traceable in each internode (one extending down from either margin of each leaf-base), which

will terminate in the axils of the leaf-pair below, on either side of each mid-point, as in *Vinca rosea*. In cases where the insertion width is just equal to one quarter of the circumference, the four contour lines descending from any leaf-pair will 'pick up' (become continuous with) those arising at the node below. Hence the contour lines in this case will seem to continue uninterruptedly from internode to internode. If more than two leaves are present at each node, as occurs not infrequently on individual branches of plants normally having but two, the surface pattern exhibits a corresponding modification, as can also be well seen in the younger internodes in *Hippuris vulgaris*.¹

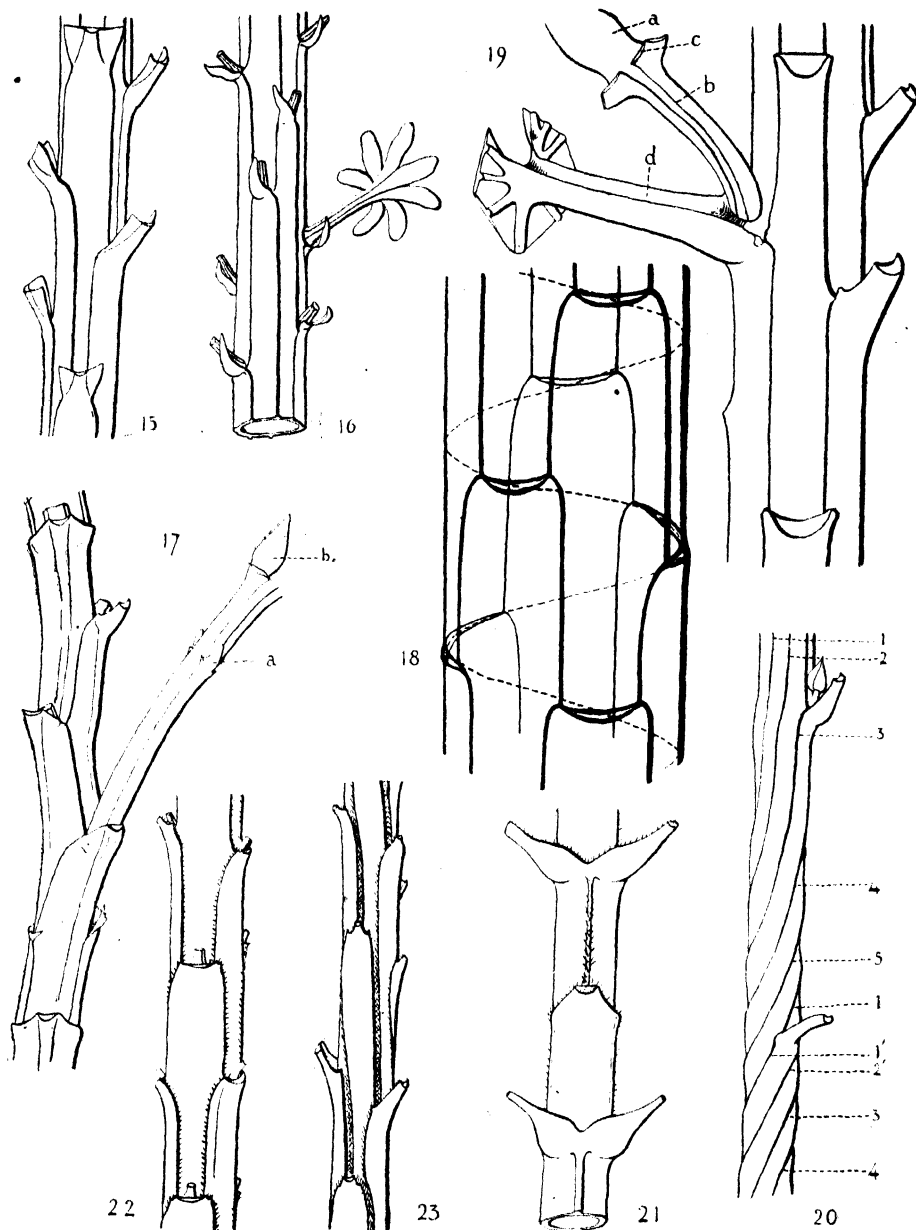
Where the leaf arrangement is spiral the number of contour lines in each internode and the number of internodes through which they run is similarly determined by the leaf-divergence and the relative leaf-insertion width. We may take as an illustration the most frequently occurring arrangement, viz. the $\frac{2}{5}$ divergence. If with this divergence the insertion width approximates to $\frac{2}{5}$ of the circumference, three contour lines will be found in each internode, for every descending line will either 'pick up' a line descending from the neighbouring leaf next below, or will 'be picked up' by one descending from the next leaf above, as shown in Fig. 11. With this configuration it follows that of the two contour lines arising from each leaf-insertion, one on each side, the one will terminate in the mid-axil of a lower leaf after running through two internodes, the other not until it has traversed three. If the individual is one with a right to left orientation (as shown in Fig. 11), then the right edge line will be the long one and the left the short. Conversely, in the reverse orientation the line from the left edge will be long and will 'pick up', that from the right edge will be short and will 'be picked up' by its neighbour. With the same leaf-divergence, but with an insertion occupying less than $\frac{2}{5}$ but more than $\frac{1}{5}$ of the circumference, there will be no actual 'pick-up', and hence five distinct contours will be traceable in each internode. For instead of a fusion line terminating in mid-axil, we shall now have two separate lines ending in each axil on either side of the mid-point, as occurs in *Calystegia* (see Fig. 19). If, still with the same divergence, the insertion is precisely $\frac{1}{5}$ of the circumference, these five lines will appear continuous from internode to internode, as described in a previous case.

But the biological pattern thus traced is not generally a strict rectilinear design. There is often some degree of convergence or curvature in

¹ It must be understood throughout this account that where biological relations are expressed in fractions, these fractions represent an approximation and not mathematical exactitude. No doubt a certain amount of 'give and take' occurs between any leaf and its neighbours. This is of no consequence from the point of view of the general principles here under consideration. All that matters is that this biological 'give and take' is adjusted so that the final result (i. e. the sum of the fractions estimated in relation not to an abstract vertical axis but to the axis of biological symmetry) is an integer. For if it be acknowledged that the leaves are decurrent, then the course of the cell files and not precise measurement will be our means of determination.



FIGS. 11-14. Diagrammatic schemes to represent phyllotaxis; the axis viewed as if split longitudinally and spread out flat. 11, *Veronica hederacifolia*, leaf-divergence $\frac{2}{3}$, leaf-insertion approximately $\frac{2}{3}$. 12, *Lopezia coronata*, leaf-divergence $\frac{2}{3}$, leaf-insertion approximately $\frac{1}{3}$; the pattern shows a lateral skew. 13, *Reseda odorata*, flowering axis; bract-divergence $\frac{2}{3}$, bract-insertion approximately $\frac{1}{3}$. 14, *Viola tricolor*, leaf-divergence $\frac{2}{3}$, leaf-insertion approximately $\frac{1}{3}$.



FIGS. 15-23. 15, *Reseda odorata*, vegetative axis; leaf-divergence $\frac{2}{3}$, leaf-insertion between $\frac{1}{3}$ and $\frac{2}{3}$. 16, flowering axis of the same, leaf-divergence $\frac{3}{4}$, leaf-divergence approximately $\frac{1}{2}$; pedicel showing the sepal extensions. 17, *Viola tricolor*, showing contours of bract and sepal extensions. *a*, bract; *b*, posterior sepal (the appendiculate portion has been cut away). 18-20, *Calystegia dahurica*. 18 (diagrammatic), stem viewed as if transparent, phyllotaxis as in *Reseda* (Fig. 15); 19, *a*, unopened flower-bud; *b*, contour lines from the bracts which have been cut away at *c*; *d*, channelled petiole; 20, after torsion. 21, *Epilobium parviflorum*; cotyledons glabrous, hypocotyl ridge without hairs, leaf-petioles ciliate, internodes with ridge line surmounted with hairs. 22-23, *Lopezia coronata*, showing the leaf-extension edges flanked with a line of hairs. 22, lower region of the stem, leaves opposite; 23, upper region, leaves spiral.

the outlines, or the whole may have a slight lateral skew. Such departure from an exact geometrical form may give rise to a deviation from the ordinary pattern, as is seen in *Lopezia coronata* (see Figs. 12 and 23). Here, although we have (as it seems) a $\frac{2}{5}$ divergence in the upper part of the shoot, and an insertion width of $\frac{1}{5}$ giving the expected five contour lines in each internode, these lines do not continue uninterruptedly down the stem, but terminate in the axil, some in the median line, some to one side of the axillary bud. Of the two lines arising at each insertion the shorter one, after being 'picked up' at the start by one descending from the neighbouring higher leaf, continues through three internodes; the longer one descends through five, 'picking up' one from a neighbouring lower leaf after it has traversed two (see later, p. 153 and Fig. 12).

With a very wide leaf-insertion, as in *Viola tricolor* and allied forms, where it approximates to $\frac{3}{8}$ of the circumference, the divergence being again $\frac{2}{5}$, only two contours appear in each internode, the two formed by each leaf extending through only one and two internodes respectively (see Figs. 13 and 17). In the flower spike of Mignonette (*Reseda odorata*) we have a transition from a $\frac{2}{5}$ to a $\frac{3}{8}$ divergence. The bract-insertion being $\frac{1}{8}$ of the circumference, we find the same uninterrupted continuity of the lines (here eight in number, see Figs. 14 and 16) as in the case of $\frac{1}{5}$ insertion with a $\frac{2}{5}$ divergence (see above). Contour relations of a like nature are also observed in the ultimate branches of the shoot axis, i.e. in the individual flower stalks. In *Viola* and *Calystegia* this is particularly clearly shown (see Figs. 17 and 19). Two bracts, large in *Calystegia*, quite small in *Viola*, accompany the flower, and are placed a considerable distance up the full-grown flower stalk. In both plants contour lines starting from the bract insertions are traceable to the base of the pedicel. As the portion of the stalk above the bract level elongates, similar lines due to the outer sepals become visible in this region. In *Reseda odorata* the pedicels show longitudinal ridges and furrows, the number always corresponding with the number of sepals, which varies from four to as many as eight (see Fig. 16).

Among Monocotyledons it is less easy to obtain the kind of evidence required for our present purpose. In this class the production in the seedling of a well-developed hypocotyl or in the adult plant of an elongated leafy shoot with a length of internode and width of leaf-insertion offering favourable conditions for the exhibition of those features which are easily demonstrated in Dicotyledons is exceptional. In the shoot axis of certain families (e.g. Commelinaceae, Gramineae, Dioscoreae), however, these conditions are fulfilled, and here we are able to see that the surface configuration depends upon the same relations as in Dicotyledons.

In the Coniferae, on the other hand, there is abundant evidence that these relations hold, as, indeed, we might expect in view of the essential similarity of arrangement and development in this group and in Dicotyle-

dons. In many Conifers, in fact, the leaf is so obviously decurrent that it is invariably described as such.

Enough has now been said to show the general nature of the evidence upon which the view here advanced is based. It has been made clear that as regards certain definite surface patterns which are visible on the shoot axis, the form of the quasi-geometrical areas which go to their make-up is the outcome of the particular leaf-divergence and leaf-insertion width. Also that the 'direction of fit' of these areas corresponds with the orientation of the leaf-spiral—that it is, in fact, merely another outward expression of the same internal relation. These facts are patent to the eye, and their significance can scarcely be disputed. They lead directly to the conclusion that in Spermatophytes the shoot axis consists throughout of an axial core enveloped in a covering of tissue of foliar origin—the leaf-skin. I have used the word 'skin' with intent, for the reason that it does not connote any precise number of cell layers. For although the evidence adduced relates only to the first hypodermal layer in addition to the epidermis, it may be premature at this point to assume that in no case does any deeper layer give indication of these boundaries.

By the downward extension of its tissue the leaf is braced against the stem, and in this way we may suppose obtains a better *point d'appui*, an arrangement which may have its value where the petiole has to carry a large outspread lamina. As is well known, the subcylindrical petiole of many Dicotyledons is frequently channelled on the upper side. Where this character is well marked, there may be a distinct appearance of a wrap-over of the edges, as in *Lopezia coronata*, where hairs border either rim of the channel, thus continuing in this middle region the demarcation by hairs of the *actual* (here arched-over) edges which has been shown to exist for the *potential* edges of the downward extensions, and which in the expanded lamina takes the form of a fine ciliation.

The differentiation of the vascular tissue of the leaf in the higher plants begins, as we know, in the periblem, about at the level at which the leaf is conceived as arising from the axis, and develops in both directions, upwards, keeping pace with the increasing length of the free portion, and downwards, usually for a definite distance which varies with the particular type, until it makes connexion with an older leaf-trace at a lower level. Now, although there are good grounds for maintaining that the region of transition from the arrangement typical of one member to that characteristic of another is not necessarily identical for all the tissues involved, nevertheless, the very fact that the leaf-trace pursues a downward as well as an upward course of development renders it at least not inconceivable that the more superficial tissues may do so too.

A decurrent mode of development of the Spermatophyte leaf seems to have been envisaged by Sachs, if we may judge from the diagrammatic

representation which appears in the 'Vorlesungen', Fig. 305, and is here reproduced, although no actual statement to this effect occurs in the text. The leaf-primordium is here depicted as arising from a single horizontal cell layer, which undergoes division and growth in such a

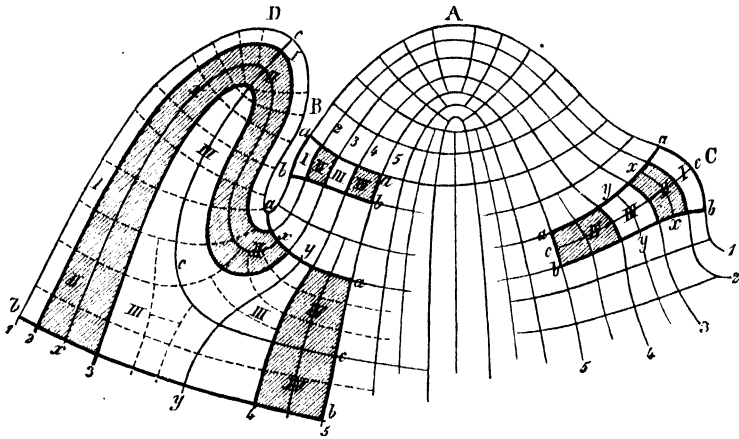


FIG. 24. Diagram to illustrate the mode in which leaves are developed from the growing-point of a Phanerogam (reproduced from Sachs's 'Vorlesungen', Fig. 305, p. 569).

manner that the resulting tissue extends downwards for some distance below the axil, in a manner entirely in accord with the view put forward in the present account.

3. COMPARISON OF THE LEAF-SKIN THEORY WITH THE CONCEPTIONS OF OTHER WRITERS.

That what in ordinary parlance we term the 'stem' is wholly or in part 'foliar' in nature is no new idea. It has existed in one form and another since a very early date in the history of Botany. In 1841—to go no farther back—Gaudichaud¹ maintained that the unit of which a higher plant was built up consisted of (1) a downwardly developed system, the root (suppressed, as a rule, except in the embryo), and (2) an upwardly developed system distinguishable into (a) a tigellar, (b) a petiolar, and (c) a laminar region, the two systems constituting a 'phyton', a series of 'phytons' making up the individual. Thus the stem as a plant member *sui generis* and distinct from a leaf was not recognized by Gaudichaud. By Hofmeister and Nägeli, on the other hand, the shoot was conceived as consisting of an axis on which were borne members of another order—the leaves. This axis, according to Hofmeister,² was not identical with the stem, but with its central region only. The outer tissue enveloping this core he held to be formed from the leaf-bases, through multiplication and

¹ Mém. de l'Acad. des Sciences, Paris, 1841.

² Vergl. Untersuchungen, 1851.

extension of the component cells in the direction of their length. A somewhat similar view was held by Nägeli, who considered it probable that the tissue of the stem surface immediately surrounding the visible leaf-base belongs to the leaf.¹ Various modifications of this conception of the dual nature of the stem have been brought forward from time to time, the most recent being the 'pericaulom' theory of Potonié, briefly outlined in 1902,² and more fully elaborated in his 'Grundlinien' in 1912, to which work the reader is referred for a more detailed discussion of the views of other writers on the subject. In the course of this survey the writer insists that his theory must be distinguished from any form of *Berindung* theory hitherto advanced, since he includes under the term 'pericaulom' not only the cortex but the deeper-lying tissue, at least in that part of the stem occupied by leaf-traces. Somewhat earlier, in 'Die Gliederung der Kaulome',³ Celakovský based his fundamental thesis that the *Stengelglied* (internode with succeeding leaf or leaves), and not the single cell nor the whole plant, constitutes the 'individual'—a view again not new (see Asa Gray, 'Structural Botany', 1881)—upon the conception that the leaves furnish a complete *Berindung* to the axis. In illustration of this argument several schematic drawings are given which, phylogenetic implications apart, the evidence here adduced fully supports (see Pl. IV, Figs. 7 a and 19). For the moment, however, we are concerned less with the points of difference between the several theories than with the fact that they have this feature in common, that they rest for the most part on metaphysical conceptions, palaeontological argument and evolutionary theory. There is little or no *direct* evidence adduced which can be said to afford any very convincing proof of the position which it is sought to establish. In the present attempt to elucidate further the morphological nature of the shoot in the higher plants, the aim has been, by setting forth certain demonstrable facts and making clear their relation to the general anatomical scheme, to furnish evidence tantamount to proof of the existence of a 'leaf-skin' in the different Spermatophyte groups, and by establishing this conception on a firm basis of fact to settle a question which ever and anon gives rise to renewed discussion, and upon which there still exists some division of opinion.

In this conception of the foliar nature of (at least) the superficial layers of the whole shoot axis, of the formation of a skin by downward extensions of the leaf-rudiments which are fused along their contiguous margins and which keep pace in their growth with the stem core, we find at least a partial solution of our original problem (see p. 135). We learn that it is to the surface character of the cotyledons that we must look for a guide to the surface character of the hypocotyl, since these two regions are morphologically one as regards their outer tissue. If the cotyledons are wholly or for

¹ Abstammungslehre, 1884.

² Ber. d. deut. bot. Gesell., xx, p. 502, 1902.

³ Bot. Zeit., 1901.

the most part free from hairs, the hypocotyl will almost certainly be devoid of hairs. If the free margins, more especially along their lower extent, are ciliate, then it is likely (though not inevitable, for the hairs peter out in some cases) that a hair line will be formed down each side of the hypocotyl. If the cotyledons are markedly hairy on the margins or the surface or in both regions, then we may expect that the hypocotyl will be hairy

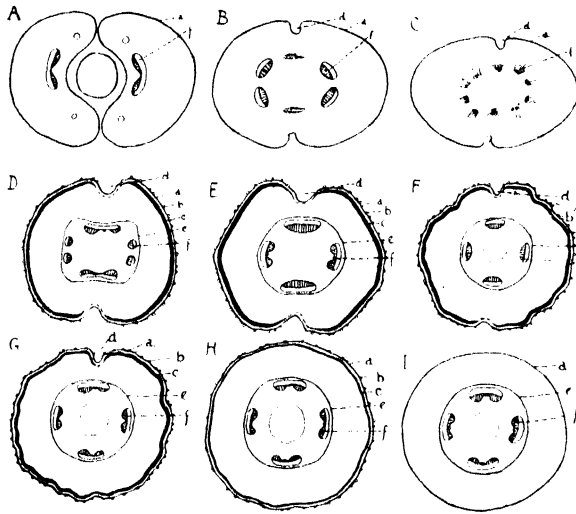


FIG. 25. A-I *Ipomoea sanguinea*, showing the appearance of the hypocotyl as seen in transverse section at successive levels from above downwards; a, epidermis; b, papillae, absent from the furrow; c, outermost layer of the cortex, containing anthocyanin; d, furrow; e, endodermis; f, fibro-vascular bundle.

all over. That is to say, where hair formation is a normal feature of the *free basal margins* of the cotyledons we may find the same character exhibited along their *potential edges*. This is as far as mere outward examination of the hypocotyl will carry us. The determination of the morphological nature of the axial core is a more difficult problem. In seedlings of the *Ipomoea* type we have a typical stem arrangement of the vascular tissue (xylem and phloem superposed) throughout the length of the hypocotyl (see Fig. 25). The opposite extreme condition in which a root arrangement (xylem and phloem alternate) is found extending up the hypocotyl to the cotyledon node has been recorded by various authors. This alternate disposition may even come about in the petioles before the cotyledon traces enter the hypocotyl as has been described by T. G. Hill¹ in *Piper cornifolium*. A similar condition has been found by Tansley and Thomas² to exist in many members of the Rhoeadales and Ranales. In a detailed investigation of the vascular tissue in the seedlings

¹ The New Phytologist, vol. iii, 1904; also Ann. Bot., vol. xx, 1906.

² The New Phytologist, vol. iii, 1904.

of Dicotyledons especially, Chauveaud¹ has shown that the transition from the alternate to the superposed arrangement may take place at any level in the hypocotyl. He further concludes from the appearances at successive levels that this transition results from the gradual disappearance, as we proceed up the hypocotyl, of the vascular elements of the root and their replacement on the alternate radii by the vascular elements of the cotyledon traces, from which observation he is led to the view that the superposed and alternate arrangements are characteristic, not of different morphological members, but of different phases of evolution. In a later paper² he formulates his idea of the construction of the plant body. He conceives it as composed of a succession of similar systems or units, each termed a 'phyllorhize', consisting of a leaf and a root, and each forming a bud from which is developed the succeeding 'phyllorhize'. These careful observations on the course of the vascular elements in the hypocotyl are of considerable interest, quite apart from this conception of evolutionary morphology which one may not be disposed so readily to accept. On the view that the existence of a stem axis has not yet been disproved, the question whether there is *continuity* or merely *contact* between the vascular elements of the trace and the root is one of prime importance. In the former case it may be urged that the region of transition cannot properly be referred either to the stem or the root; in the latter case the lower limit of the stem tissue would be defined by the interdigitating ends of the vascular strands, the *foliar* character of the external tissue being unaffected on either view. This latter point being the one with which we are here mainly concerned, it may now be well to describe the topographical features of a few illustrative cases in further detail.

4. FURTHER DETAILS OF SURFACE TOPOGRAPHY IN SOME ILLUSTRATIVE CASES AMONG ANGIOSPERMS AND GYMNOSPERMS.

Dicotyledons.

Hypericum calycinum. Leaves opposite. A longitudinal welt marking the line of junction of the confluent leaf extensions runs down either side of the internode, starting from the points where the leaf-blades meet and terminating in mid-axil at the next node below.

Epilobium parviflorum. Leaves opposite. Cotyledons glabrous. Hypocotyl glabrous, with a longitudinal welt on either side extending down from the points where the cotyledon blades coalesce. In the internodes of the young stem the raised contours representing the lines of fusion may be further marked by a distinct line of hairs (see Fig. 21).

Ruellia amoena (Acanthaceae). Leaves opposite. Cotyledons with a short pile on the upper surface of the petioles, otherwise glabrous. Hypocotyl and stem with two well-marked lines of hairs extending through-

¹ Ann. des Sci. Nat., Bot., t. xiii, 1911.

² Comptes rendus, t. clviii, p. 343, 1914.

out the internode from leaf junction above to mid-axil below, well developed even in the youngest internodes.

Asclepias linifolia. Leaves opposite. Internodes with two conspicuous lines of hairs or with one line broad and well defined and the other narrow or even wanting. In the last-mentioned case the positions of the one line in successive internodes lie on a continuous spiral, as in *Stellaria media* (see below), though occasionally the direction is observed to become reversed even in unbranched stems standing erect.

Stellaria media. Leaves opposite. Stem glabrous except for a single well-defined line of hairs extending from one of the two leaf junctions at each node to the mid-axil of the leaf immediately below. A cross-section of the middle region of the internode shows a ring of four vascular bundles symmetrically disposed. The two in line with the midribs of the leaf-pair above are small; the alternate pair occupy a more extended arc. It is exactly opposite one (or sometimes both) of these larger double bundles that the hairs arise (see Fig. 10). In each successive internode this line shifts through a quarter of the circumference, always in the same direction, so that it traces a spiral round the axis. The direction of the spiral may be either to the right or to the left, and may be in opposite directions in different branches on one individual. Among most of the plants examined the lowest internode on the chief axes was without this hair line; sometimes this was the case also with the next one or more. Once having appeared, however, it continues almost without exception uninterruptedly up the stem. Now and again an internode may be found with a second hair line, sometimes weaker, sometimes as well marked as its fellow, starting from the other (opposite) leaf-junction. Attempts to relate the occurrence of two lines of hairs with any other external morphological feature proved unsuccessful. So far as appeared, the demarcation of the second leaf-extension fusion line in any internode was not directly related to the degree of vigour of development, symmetrical or otherwise, of the axillary buds either at the node above or below. In other words, no expectation could be formed as to when two lines would be found. There is, however, some indication that a second hair line is more frequent in plants grown in dry soil and fully exposed to the light—a conclusion quite in accord with that arrived at in other cases. Certain other relations of the single, or of the more pronounced line if two are present, appear to be more strictly laid down. When this hair line appears on the lowest internode of a vegetative lateral axis it always occurs on the adaxial side, but the direction which the spiral will then proceed to take can only be ascertained by inspection of the next (second) internode. In the flowering region, where the axillary bud of both leaves usually develops, the main axis (the central flower stalk of each cyme) becomes pushed at each successive node to one side (a displacement which does not occur when both

axillary buds develop in the vegetative region) and curves outwards and downwards on the side of the stem opposite that on which the hair line is formed in the internode immediately below. A well-marked line of hairs extends along the whole length of this flower axis on the inner (convex) side, i. e. the side which faces the geometrical centre of the whole system, and arises from the insertion of the posterior sepal whose edges lie outside its neighbours, and whose insertion width is so narrow that the two lines which we might expect to find apparently come into contact and form the single track which we see. On both the axillary axes which spring from the same node the hair line develops on the adaxial side, as has been described for the case of the lateral branches in the vegetative region. These relations have been observed also by Lundström,¹ who accounts for the suppression of the second line of hairs in the internode beneath the flowering node on the supposition that it would be useless in this position and hence is not formed. But this argument does not meet the case in the vegetative region. It would rather seem that hair formation is the outcome of a certain physiological condition which is not usually attained in the basal internodes, but is reached and maintained at higher levels, and may even now and again exceed this normal limit and so lead to the formation of a second hair line. The direction of shift of the single line is not apparently predetermined, but is the result of a combination of causes affecting equilibrium at a particular moment.

Upon comparing individuals growing in different situations, it becomes evident at once that the general uniformity of hair distribution characteristic of the axial surfaces is not manifested in the free region of the sepals, which varies greatly in degree of hairiness. In some plants the whole of the surface exposed in the unopened bud is hairy; in other individuals it may be difficult to find a single hair. But even though there be but one on the posterior sepal itself, the hair line marking the boundaries of its extension down the pedicel is as conspicuous as in those cases where the unopened bud is hairy all over. The production of hairs on the free surface of the sepal takes place in response to the particular conditions prevailing during the development of the axis, and has no hereditary significance. The hair line marking the boundaries of the sepal extensions is an inherited feature related to a definite morphological configuration, and makes its appearance as soon as the internode has an appreciable length.

The general occurrence in *Stellaria media* of the single well-marked hair line in the internodes suggests (1) that an inequality of growth analogous to, if not identical with that which leads to the transition from an opposite to a spiral leaf-arrangement in so many species, or which finds expression in the circumnutation of the shoot tip, is inherent or invariably becomes set up in this species; (2) that this inequality is reflected along the lines of junction

¹ Nova Acta Reg. Soc. Sci. Upsaliensis, 1884.

of the leaf-extensions, although it does not cause any obvious displacement of the leaves themselves ; and (3) that exceptionally it may be sufficiently lessened temporarily as to do away with any outward effect of asymmetry and hence the two equal hair lines. This interpretation of the *Stellaria* configuration appears to be more consonant with the facts than the explanation offered by Eichler,¹ who considers that although the leaves are opposite and decussate, yet, since one member of the pair begins to develop a little earlier than its fellow, we should regard them as in fact spirally arranged with a leaf-divergence of $\frac{1}{4}$. To which conception we must presumably add the idea of a one-sided effect on the leaf-extension in order to account for the general absence of the hair line from one edge of the leaf-insertion.* A similar one-sided disposition of the hair line is sometimes to be observed in seedlings. In some individuals of *Cosmos bipinnatus*, for example, a distinct line of hairs was present on one side of the hypocotyl, but except for two or three immediately beneath the point where the free margins of the cotyledons coalesced, the opposite side was destitute of hairs. Yet it surely would be somewhat fanciful on this ground to apply the conception of a $\frac{1}{4}$ divergence to the cotyledons. In other species showing a difference between the two sides of the axis, the normal condition may be one in which this inequality is much less pronounced, as in the case where two hair lines commonly occur but the one is much weaker than the other (observed in seedling plants of *Bidens ferulacifolia*, D.C.). It has seemed necessary to dwell at some length on the case of *Stellaria*, since a mere statement of the facts without further analysis would make it appear that this configuration was not amenable to interpretation on the theory of the leaf-extension as here set forth.

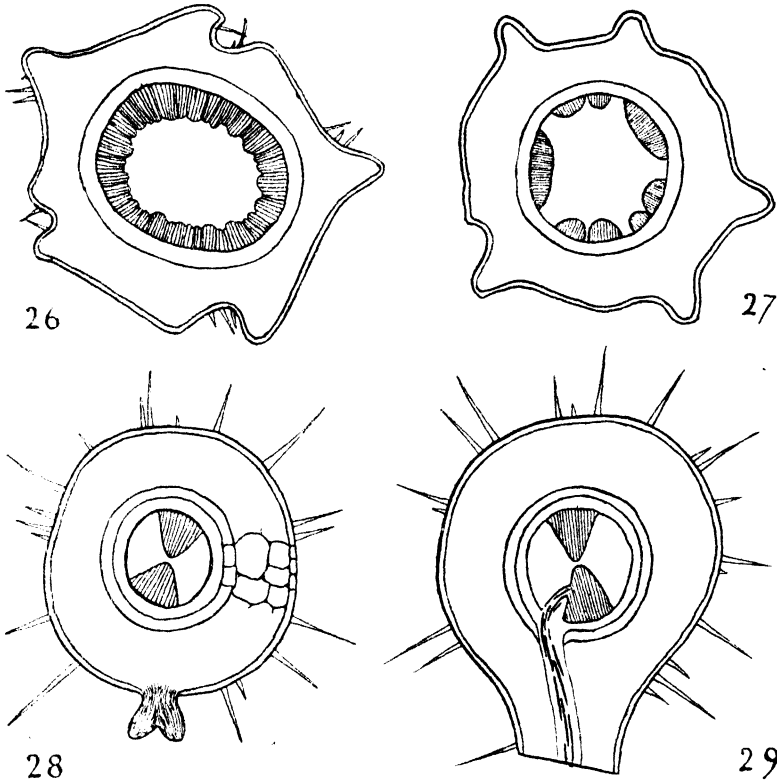
Certain hairy-leaved species of *Veronica*. In *V. hederacifolia*, *V. arvensis*, and *V. polita*, as well as in the familiar case of *V. Chamaedrys*, a definite hair pattern is exhibited when the plants are growing under certain conditions. That this fact has hitherto only been generally recognized for *V. Chamaedrys* is due no doubt to the circumstance that in this species the hairs are of one kind, and that the rest of the surface is nearly or quite glabrous ; whereas in the other species named the hairs are usually of two kinds, the one long, needle-like, and spreading, the other short and closely set, forming a sort of pile. Along the line of fusion of the leaf-extensions we find a tract covered with pile, but with few if any of the long hairs which are abundant over the rest of the surface. This appearance is particularly clear in *V. hederacifolia*. In this species the cotyledons and hypocotyl are frequently glabrous. Under suitable conditions, however, hairs develop on the margins of the petioles and extend down opposite sides of the hypocotyl in two vertical lines, petering out, it may be, before reaching the root, the rest of the surface being desti-

¹ Blüthendiagramme, II, p. 17.

tute of hairs. Under conditions still more favourable to hair formation, on the other hand, the whole surface may be covered with hairs. It becomes evident, in fact, that the lines of fusion of the cotyledon extensions remain invisible, or are brought out, or obliterated, according to the extent to which the conditions happen to be favourable to hair development. In the four species of *Veronica* named above, the first leaves are always opposite. This arrangement may persist up the stem (*V. arvensis*, *V. Chamaedrys*), but in the flower spikes, if not before, the arrangement becomes spiral with a $\frac{2}{3}$ divergence. In *V. hederacfolia* and *V. polita*, where the flowers are solitary and axillary, the spiral arrangement is assumed at an earlier stage. Coincident with this change in the leaf-arrangement we find in individuals in which the hair lines are apparent a corresponding change of pattern. In place of two lines to the internode, both extending only from one node to the next, we now have a three-line pattern resulting from a 'pick-up' along the lines of fusion of the kind already described (see p. 140 and Fig. 11). Of the two lines arising at each insertion one now descends through two internodes and the other through three, the one from the right or from the left edge being the longer of the two according to whether the individual is one in which the spiral proceeds from right to left or from left to right. In *V. hederacfolia* a well-defined hair line is also traceable along the whole length of the flower stalk, marking the line of fusion of the extensions of the two postero-lateral sepals, and ending in the mid-axil of the subtending leaf where it meets the hair-line on the main axis terminating at the same point.

Lopezia coronata. Leaves opposite below, becoming spiral above, upper surface slightly hairy towards the base, margins finely ciliate. Here again under the appropriate conditions two lines of hairs are developed on the hypocotyl, descending from the points where the free margins of the cotyledons coalesce (see Fig. 4). A decussate arrangement of the leaves is continued for perhaps fourteen or fifteen nodes, a difference in the level of insertion of the two members of a pair, which is slight to begin with, becoming very considerable higher up. The internodes in this lower region show two contour lines flanked by lines of hairs which are absent from the surface elsewhere (see Fig. 22). Some interest attaches to a case in which an irregularity in the leaf arrangement occurred in view of the conception of the 'potential' edge which has been given above (p. 138). In the specimen in question two leaves were borne at the first and second nodes, a slight difference in the level of insertion being noticeable in the latter case; at the third node only one leaf was formed, while three appeared at the node above. It was evident that one of the pair belonging properly to node 3 had become fused with the axis in its upward development, and hence only became free at the node above its true insertion. In consequence of this disarrangement, the leaf which was carried from node 3 and one

of those properly belonging to node 4 were placed so near together that a deeper fusion of tissue than usually takes place may be supposed to have existed, with the result that the line of contact between them no longer possessed the character of a 'potential' edge and hence produced neither hairs nor contour ridge. In the upper region of the stem, as already described (p. 143), the leaf-divergence is $\frac{2}{3}$ and the leaf-insertion width perhaps a trifle more than $\frac{1}{3}$ of the circumference. The contour lines diverge



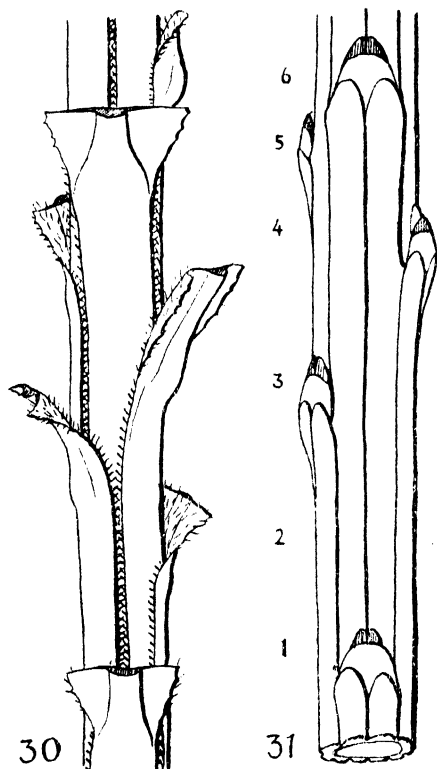
FIGS. 26-29. 26, *Lopezia coronata*, stem in transverse section showing the contour lines of the leaf-extensions as eight ridges flanked with a line of hairs. 27, *Calystegia dahurica*, showing similar ridges without hairs. 28-29, *Antirrhinum Orontium*, hypocotyl in transverse section showing an early and a later stage in the development of an exogenous adventitious bud.

slightly from a straight course, so that of the two terminating at each node one ends in mid-axil, the other to the side and close to one of the minute stipules. The 'lie' of the pattern is therefore not truly in line with the axis, but slightly askew. Owing to this cause and to some slight departure from a parallel course, the two contour lines from each leaf-insertion now run through three and five internodes respectively (see Figs. 12 and 23) instead of only through two and three internodes, as is the case with the same arrangement when the fusion lines run parallel to the axis (as shown in Fig. 11). As the hair lines flank each ridge and as contiguous hair lines

become merged we get a pattern of eight ridges and five hair lines to the internode (see Figs. 12 and 26).

Lobelia fulgens. Leaf-divergence $\frac{2}{3}$, leaf-insertion width about $\frac{2}{3}$ of the circumference. The contour relations here are the same as those described for *Veronica*, but the stoutness of the *Lobelia* stem and its deep red colour, against which the colourless hairs are particularly conspicuous,¹ render

it specially favourable material for observation. From the broad leaf-insertion there extends down the stem on either side a distinct flange or wing (see Fig. 30), along the margin of which, under certain conditions, is formed a line of hairs. When this is the case, this line of hairs is found as high up the stem as it is possible to examine the internodes, the rest of the surface being completely glabrous. As the stem becomes older a further development of hairs may take place in response to altered conditions, and this may result in a general flooding, as it were, of previously smooth regions with hairs. Under quite other conditions no hairs at all may be formed, and the wing itself may be little pronounced. But again we may stress the point that the fact that the surface *may* remain free from hairs and almost smooth or *may* show a blurring of the original pattern is beside the mark. Our aim is to prove that certain boundaries exist, and that under suitable conditions they become clearly defined.



FIGS. 30-31. 30, *Lobelia fulgens*, leaf-divergence $\frac{2}{3}$; leaf-insertion approximately $\frac{2}{3}$, with decurrent wing margined by hairs. 31, *Cytisus purgans*, leaf-divergence $\frac{2}{3}$, leaf-insertion approximately $\frac{2}{3}$; the number of contour lines is doubled owing to the additional one formed in line with the midrib (nodes numbered from below upwards).

Convolvulus arvensis, *Ipomoea coccinea*, *Ipomoea sanguinea*, *Calystegia dahurica* (Convolvulaceae). In this family contour ridge lines are a very characteristic and conspicuous feature, and are traceable despite any degree of torsion in all regions of the stem (see Figs. 19 and 20). In *Calystegia*

¹ As the anthocyanin in this species occurs in the epidermal layer this distribution is remarkable. We have here, as we shall have occasion to note again later elsewhere, a sharp delimitation within tissue accompanying different development. At an early stage *all* the epidermis is coloured, but as hairs and guard-cells become differentiated, the colouring matter disappears from these specialized cells.

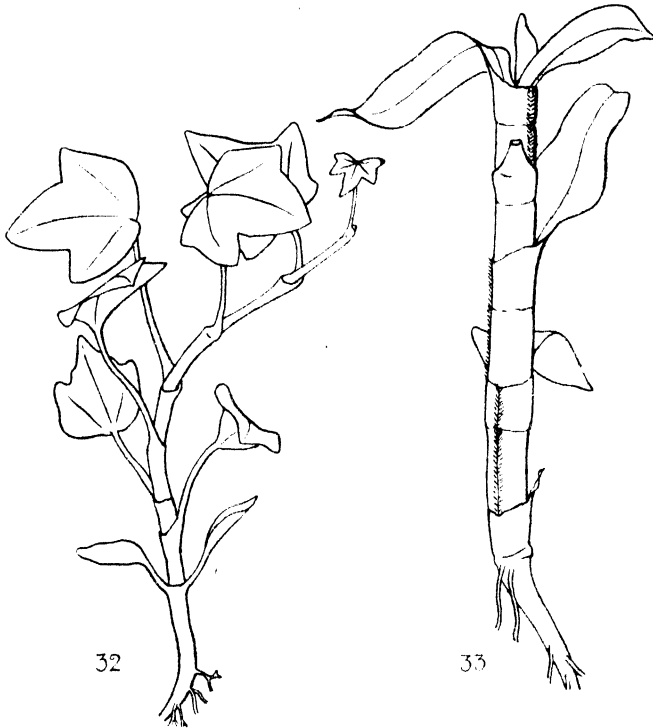
dakurica, which we may take as an example, the leaf-divergence is $\frac{2}{3}$ and the insertion width between $\frac{1}{5}$ and $\frac{2}{5}$, hence, as explained above (p. 140) and as shown in Figs. 18 and 19, no actual 'pick-up' occurs. Each contour ridge runs a separate course through two or three internodes according as it starts from one side or other of the insertion, and the two adjacent lines from neighbouring leaves (one from the right edge and one from the left) terminate in each axil. We thus get five contour lines in each internode. The stem is coloured with anthocyanin, and this is ordinarily confined, as in the seedling, to the layer beneath the epidermis, where most of the chlorophyll is also located. A cross-section through the middle of the internode (see Fig. 27) shows a ring of five vascular bundles, each extending through a considerable arc midway between two ridges. The second and succeeding layers of the cortex become converted into collenchyma, and the whole central tissue of each ridge may be thus thickened, but the outermost layer containing the anthocyanin remains unchanged. At the crest of the ridge, however, i. e. along the line of fusion of the leaf-extensions, there is frequently a gap of one or two cells in the anthocyanin ring, just as there occurred a similar break in the furrow (fusion line of the cotyledon extensions) of the hypocotyl in *Ipomoea* (see above, p. 138, and Fig. 25). When this is the case these colourless junction cells may undergo collenchymatous thickening as well as those lying deeper. The same 'potential edge' character is thus common to the furrow of the hypocotyl and the ridge lines on the stem. The axillary flower stalk is marked by similar contour ridges, due in the lower region to the downward extensions of the two bracts and in the upper portion to those of the outer sepals (see Fig. 19). The petioles are stout and channelled, and their insertion, owing to their angular outline, gives rise to special trace lines which form a subsidiary pattern within the larger configuration. Such subsidiary patterns are not infrequently present when the petiole is four-angled, as is seen in *Rivina humilis*, especially if the leaf is large and compound, as e. g. in *Phaseolus multiflorus*.

Viola tricolor. Leaf-divergence $\frac{2}{3}$, leaf-insertion width about $\frac{3}{5}$. The stem shows two well-marked contour ridges in each internode. Of the two arising from each insertion one descends through only one internode, the other through two (see above, p. 143, and Figs. 14 and 17). As is the case in *Calystegia*, the flower stalk exhibits ridge lines due to the two bracts, minute as they are, which are placed some distance up the pedicel. As the region above the bract level elongates, similar lines arising from the posterior sepal can be traced passing between the bracts (which are not near enough together to furnish a 'pick-up') along the whole length of the flower stalk.

Reseda odorata. Leaf-divergence in the vegetative axis $\frac{2}{3}$, leaf-insertion width between $\frac{1}{5}$ and $\frac{2}{5}$. General scheme similar to that of *Calystegia* (see

above, p. 140, and Fig. 15). In the flower spike the arrangement changes to a $\frac{3}{8}$ divergence with a bract-insertion width of $\frac{1}{8}$, the eight contour lines thus produced being continued uninterruptedly along the axis (see Figs. 13 and 16). On the individual flower stalks from five to eight lines demarcating the downward extensions of a corresponding number of sepals are plainly visible.

Cytisus purgans. Leaf-divergence $\frac{2}{5}$, leaf-insertion width about $\frac{1}{5}$. With this combination we should ordinarily expect a continuous five-line configuration, but here we find ten lines (see Fig. 31). This doubling of the



FIGS. 32-33. 32, *Hedera Helix*, seedling plant; a dicotyledon type with circular leaf-insertion. 33, *Tinantia fugax*, a monocotyledon type with a conspicuous line of hairs along the line of fusion of the edges of the tubular sheath and of the leaf-extension; cotyledon apex withered.

contours is due to the fact that in this species the free portion of the leaf is reduced to two small paired membranous flaps, and the five additional lines arise at their point of junction. The appearance that these membranous flaps would readily peel off, bringing away a longitudinal green strip beneath them, is suggestive of that actuality which in its more pronounced form, when the leaf is obviously decurrent, is universally recognized.

Hedera Helix. Leaves spiral. In the seedling it can be seen that the leaf has an insertion which completely embraces the axis, hence each internode is entirely covered with the downward extension of a single leaf (see

Fig. 32). This example is of particular interest in view of the strict comparison it affords with the monocotyledonous condition as exemplified in the Gramineae, Palmae, Commelinaceae, and other families.

Monocotyledons.

Tinantia fugax, *Commelina coelestis*, *Tradescantia fluminensis*, *Zebrina pendula* (Commelinaceae). The first three plants furnish perhaps as striking an illustration as can be found of the demarcation of the fused edges of the leaf by a line of hairs. In each of the genera the leaf-insertion embraces the axis. Above the node level the leaf forms a short tubular sheath, surrounding but free from the axis, before it expands into the free lamina, the basal margins of which are ciliate. On the side of the sheath which represents the fused edges a well-marked line of hairs is present, which, except in *Zebrina pendula*, is continued past the node and down the whole length of the internode beneath, until it terminates to one side of the mid-point in the axil of the next leaf below (see Fig. 33). Elsewhere the surface is glabrous except at the insertion level in *Zebrina* where hairs occur especially over the spot¹ where the axillary bud or a root will break through later. In the flowering region, as is so often the case also in Dicotyledons, the plant may become generally hairy (*Tinantia*). In the first two genera named above² there are no hairs on the well-developed hypocotyl, a fact which becomes comprehensible when it is seen that the margins of the cotyledon sheath, unlike those of the leaves, are without hairs.

Gramineae. In the adult plant it needs no very close examination to see that the base of the split leaf-sheath is not the lower limit of the external tissue of the leaf, and that the 'skin' or outer surface, now fused with the axis, is continued down for one internode below the level of insertion; while in regard to the morphological nature of the structures present in the seedling the view arrived at by Sargent and Arber,³ as the result of a detailed study of this family, is not only entirely compatible with the position here taken up but affords it a certain measure of support, notwithstanding the additional complications which render the elucidation of the Gramineae particularly difficult. For it may well be that such a process of fusion as is envisaged in the present account preceded phylogenetically the further fusion which these authors hold to have occurred through close juxtaposition of the cotyledon stalk with the hypocotyl and thus to have formed the 'mesocotyl'.

Dioscorea quinqueloba. The leaf arrangement and insertion, and the resulting surface pattern, are so similar to the configuration seen in the

¹ Arising presumably as the direct outcome of pressure or strain set up by the underlying protuberance.

² Seedlings of the two other types were not obtainable.

³ Ann. Bot., vol. xxix, 1915.

Convolvulaceae that the description which has been given for *Calystegia* may be taken as applying equally to the present case.

Gymnosperms.

Coniferae. The obviously decurrent character of the leaves in the adult shoot of many species has already been mentioned. In a seedling plant of Juniper, for example, it is evident at a glance that between successive whorls of three leaves the surface of the stem proper is nowhere exposed. The three longitudinal contact lines seen in each internode extend throughout its length, terminating in the mid-axils of the whorl below. In an *Araucaria* seedling a 'pick-up' pattern after the manner of a Dicotyledon is especially conspicuous, while in *Pinus maritima* (Fig. 8) and *Picea orientalis*, Carr, as mentioned above (p. 138), we have a hypocotyl with ridges and furrows corresponding in number with the cotyledons.

5. EVIDENCE FROM THE PTERIDOPHYTA.

A discussion of the question whether the leaf-skin theory is generally applicable to the Pteridophyta lies outside the scope of the present paper. It will not however be out of place to call attention to certain features in one or two types which suggest that the same relation may exist among Vascular Cryptogams as has been found to run through the Spermatophyta.

Equisetum. The sequence of cell divisions accompanying the development of the leaf in *Equisetum* is shown in the accompanying figure, which is reproduced from Sachs's 'Lehrbuch' (Fig. 273, first edition; Fig. 279 a, second edition). A careful scrutiny of this illustration brings to light the fact that the development of the successive segments cut off from the same side of the apical cell follows a different course in alternate segments. In one rapid divisions take place in an anticlinal and a periclinal direction which soon result in the production of a slight bulge (see the point marked *b* on the right-hand side of the figure). In the segment next above, after the first anticlinal divisions have occurred, development for the time being comes to a stand. It is further evident from the drawing that a leaf is developed from each of the rapidly dividing segments, and that the *whole* product of one such segment (i. e. the cell group between any two segments which after one or two divisions remain for a time quiescent) takes part in its formation. As the cells forming the bulge or leaf primordium undergo further division, one of their number situated at the apex takes on the functions of an apical cell, and it is by the segmentation of this cell that the elongation of the leaf in an upward direction is continued. The cells forming the lower part of the bulge, i. e. the residual cells derived from the same original segment, also continue to divide and furnish the surface layer of the lengthening internode (see cells marked *r*). At a much later period one of the group of cells (marked *i*) derived from the alternate primary segment

resumes activity, and gives rise to a new apical cell which divides to produce the axillary bud. Thus, in the envelopment of the internode with a leaf-skin through the extension below the node level of the lowermost cells of the leaf primordium, the scheme of leaf development in *Equisetum* is wholly comparable with that described above for a Spermatophyte. In both, these extensions cover and are fused with the internode below the node at which the leaf separates from the axis, and keep pace with the elongation of the enclosed core. *Equisetum* is the type which Hofmeister

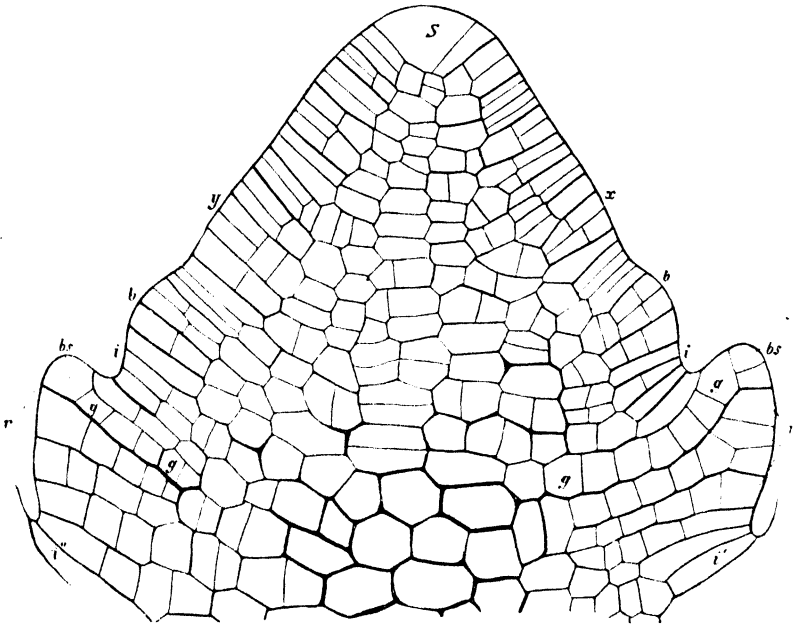


FIG. 34. Longitudinal section through the stem apex of *Equisetum telmateia* (reproduced from Sachs's 'Lehrbuch', 1st ed., p. 336). *x, y* indicate the highest, youngest rudiment of an annular swelling which will develop into a leaf-sheath; *b, b*, the same older; *bs*, apical cells of a still older leaf-rudiment; *g*, rows of cells from which the vascular bundles are derived; *i*, the lowest layers of cells of the segments; ¹ *r*, the rudiment of the cortex of the internode; *s*, apical cell of stem.

takes in illustration of that *Berindung* of the stem which, he considered, probably occurs in all leaf-bearing plants from *Chara* upwards. He was, however, unable to furnish evidence in support either of this general pronouncement or of the more definite statement that the stem is represented by the pith alone and that all the tissue external to it is to be regarded as foliar in character, and based his view on phylogenetic considerations and grounds of general probability.²

¹ It is of interest to note in the present connexion that in the legend accompanying this figure in both the first and second editions it is stated of the cells indicated by the letter '*i*' that they take no part in the formation of the leaves, but that in the legend to the same figure in De Bary's *Vergleichende Anatomie* this sentence does not appear.

² See *Flora*, 1863, p. 173.

Tmesipteris and *Psilotum*. In *Tmesipteris* and *Psilotum* the appendages of the stem are by general consent regarded as leaves, and in both genera they have been described as decurrent.¹ In *Tmesipteris* they are obviously so, and the envelopment of the axis is as self-evident here as in many of the Conifers. In *Psilotum* the very minute subulate leaves are seated on the ridges of the angled stem and their insertion occupies an insignificant fraction of the circumference in the adult axis. They are composed of epidermal and cortical tissue and contain no vascular strand. In *P. triquetrum* they are spirally arranged, though the divergence varies, as may happen, as we know, in a Spermatophyte. In slender shoots, which are generally markedly three-angled, the divergence is plainly $\frac{1}{3}$. In stouter axes, which are roughly five-sided, it may equally plainly be seen to be $\frac{2}{5}$, and transitions can be found from one arrangement to the other. The ridges appear clearly as downward extensions of the leaf-insertion. When the divergence is $\frac{1}{3}$ they descend past the level of insertion of the next two leaves before they strike one (the third) vertically below, which gives rise to a fresh ridge section in line with the one just terminated. In the case of a $\frac{2}{5}$ divergence, each ridge section extends past the insertion level of four successive leaves before striking one which terminates it. The ridges are paler in colour than the rest of the surface, and like the free portion of the leaf are destitute of stomata, which are scattered thickly and irregularly over the broad intervening tracts (the flat sides). In *P. flaccidum*, where the leaves are arranged alternately on the edges of the flattened axis, the same relation holds, stomata being absent from the marginal tracts descending from each leaf-insertion to the next. These anatomical relations raise a question of considerable interest from the present point of view. Does the width of the *Psilotum* leaf where it becomes free represent the real width of the extension at the level of origin, as we find to be the case in the higher plants, however small the free portion of the leaf may be (see, e.g., Fig. 31), or are the free structures much-contracted tips, the downward extensions of which are nevertheless broad enough to meet and unite laterally? In other words, do we see here a surface which is only partially foliar in character, a condition which so far as appears does not occur in the Spermatophyta, or have we to suppose that a contact line runs down the middle of the stomata-bearing tract, passing now between and now through stomata indiscriminately, and that the fusion is so complete (or deep) as to leave no visible trace? Among Spermatophytes it is rare to find the insertions so small in proportion to the whole circumference that those belonging to the several leaves in one cycle of the spiral, if aligned at one level, would obviously not meet round the axis, but the succulent stems of *Senecio articulatus* and *S. anteuphorbium* furnish cases in point.

¹ See A. P. W. Thomas, Proc. Roy. Soc., vol. lxix, p. 349, 1902, and G. M. Sykes, Ann. Bot., vol. xxii, p. 73, 1908.

The circumference of the axis here is often far too great to be encircled in this way by the leaf-bases. But we find contour lines starting from the insertion which spread out sideways far enough to meet those descending from neighbouring insertions, so that it becomes evident that the width of insertion cannot be taken as an invariable guide to the width of the descending extension since a sharp contraction may occur at the node. These contour lines in *Senecio* as well as the line from the midrib are free from stomata which are scattered thickly over the intervening areas. On the other hand, in the succulent stem of *Euphorbia pendula*, where the leaves are reduced to non-vascular scales, there is no trace of a fusion line, and the stomata are scattered round the whole circumference, midrib line and line of fusion included. From these facts it would then be possible to argue either way in regard to the appearance presented by *Psilotum*, but the clear evidence afforded by *Tmesipteris* inclines one to the view that an enveloping leaf-skin exists also in the related genus.¹

6. THE BEARING OF THE 'LEAF-SKIN' THEORY ON CERTAIN GENETICAL PROBLEMS.

It will be clear from what has been said in the course of this account that it may be possible in a plant exhibiting a varying degree of hairiness to resolve this variable character into two components: one primary (i.e. exhibited in the earliest stages of development), constant (up to a point), fixed in position, inherited (subject to permissive conditions), because it is the expression of certain physiological conditions definitely associated with a particular anatomical configuration. Since the anatomical contours are constant, the manifestation of the character especially associated with them is constant also under a range of conditions, wider or narrower for different types and not at present capable of precise formulation. The other, adventitious, appreciably inconstant in degree, variable in position, not inherited, representing a secondary effect superposed upon the primary, a response in areas still plastic enough to respond to conditions set up at a later stage in development, and causing a flooding effect which may lead to blurring or obliteration of the inherited feature. This secondary effect is of common occurrence in the flowering region of many plants which are more or less free from hairs in the vegetative region. The production of flowers and small bracts in place of much larger foliage leaves must involve a considerable physiological readjustment, and may well be a prime cause of the general condition of hairiness which often sets in at this point.

We are in the habit of classifying the characters of organisms under the head of those which are inherited and those which are not, although

¹ The early divisions of the daughter segments of the apical cell in *Psilotum* have been followed, for some distance by Solms-Laubach, but the origin of the leaf rudiment is not shown (see Ann. du Jardin botanique de Buitenzorg, vol. iv, 1884).

recognizing that these categories are not sharply divided, for it is evident that inheritance of some characters does not take place in equal measure in different types. The hairy character affords a case in point. The conditions which lead to general hairiness in the vegetative shoot of such a form as *Matthiola incana* prevail throughout this region and throughout the plant's life, and with such uniformity that we can detect no difference between individuals. We have here an example of strict or constant inheritance. In other instances, such as some of those described above, in which the hairiness is localized, the constitution of all the individuals of the species may be alike in regard to potentiality for hair production, so that if the appropriate conditions are maintained the hairy character is always exhibited in an expected manner. But it may frequently happen that this is not the case. We have then what we may describe as conditional inheritance. The character *may* not be manifested, but if it is, it will appear in due order in relation to the general development, as is well seen e.g. in *Asclepias linifolia*, where the physiological condition existing in a lateral axis may be sufficiently different from that in the main axis for a conspicuous hair line to be formed in the one case while it is absent in the other. But in another type a more general state of plasticity may exist, so that a further development of hairs may occur without a definite time or place relation. The character then ranks among those which are fluctuating or non-inherited. With fuller knowledge classification in this manner may become unnecessary, but in the meantime the use of some terminology such as that here adopted, which indicates these distinctions, is convenient.

From this point of view anthocyanin production in the vegetative shoot may be classed with hair formation. Localization of the colouring matter in a particular tissue or layer can be observed in many species. Where there is inheritance of the 'constant' type we should expect this, though an overflow beyond these limits might be anticipated under favourable conditions. In *Lobelia fulgens*, where the inheritance appears to be 'constant', the pigment is confined to the epidermis and perhaps a cell here and there in the first hypodermal layer. Although present in such quantity as to produce a very deep coloration it is absent from the unicellular hairs (see foot-note to p. 154). On the other hand, in *Lopezia coronata*, where also the anthocyanin occurs in the epidermis, the hairs are coloured as well. In other cases, as in the uniformly coloured stem of a red-stemmed *Dracaena*, the pigment is present in the first hypodermal layer and is absent from the epidermis. Though in a particular species the potentiality for anthocyanin production may be constantly exhibited in a particular cell layer, an alteration in physiological equilibrium arising from internal causes may lead to the appearance of the pigment elsewhere. Thus in the hypocotyl of *Antirrhinum Orontium* a slight amount of

anthocyanin is usually present in the innermost layer of the cortex, and also, though to a less extent, in the outermost layer, the intervening layers and the epidermis being colourless. Now in this species adventitious buds, from one to three or four in number, are frequently formed in the lower region of the hypocotyl and develop into branches. These buds are exogenous in origin and are derived from the epidermis and first hypodermal layer (see Figs. 28 and 29). In a hypocotyl containing so little anthocyanin that outwardly it appeared green these buds, even when sufficiently developed to show two divergent lobes representing the first leaf-pair, may nevertheless be coloured a deep red in every cell, thus presenting a striking contrast with the axis on which they are borne. Here a general flooding of the whole embryonic structure has taken place under altered physiological conditions. In passing it may be noted that the presence of adventitious buds on the hypocotyl has now to be viewed from the point of view of the development, normally and not as the result of mutilation or other injury, of a branch from *foliar* tissue, which furthermore shows no differentiation in the way of affording fixed points for their origin. For there seems no reason to doubt, especially in view of the uniform degree of hairiness displayed from the cotyledon node to the 'collar', that the cotyledon skin is coextensive here, as elsewhere, with the hypocotyl axis. What is the immediate cause of these outgrowths which have no definite relation to the general anatomical configuration—the entering trace joins on anywhere to the inner face of a secondary xylem bundle by curving to one side (see Fig. 29)—is not self-evident. Possibly a clue may be found in the relation between the amount of parenchymatous and vascular tissue present in the axis. In the diarch root of *Antirrhinum* the primary xylem is extremely scanty in amount, there being only two or three small vessels at each pole. Secondary thickening sets in so early that it is a matter of considerable difficulty to obtain a section of a root in which secondary increase has not yet begun. The secondary xylem forms a solid core and is considerable in amount in proportion to the bulk of the root. In the hypocotyl also there is proportionately little parenchyma (generally two layers or at most three besides the bundle sheath), so that it remains extremely slender in spite of its considerable length. It is conceivable that these adventitious buds result from some extreme condition (? of tension) set up in the hypocotyl, which, where a larger bulk of parenchymatous tissue is present which can act as an absorber, or where the cotyledons or epicotyl grows more rapidly, becomes distributed or adjusted without the production of adventitious outgrowths.

SUMMARY OF CONCLUSIONS.

1. The surface tissue of the Spermatophyte shoot axis is of foliar origin, that is to say, the leaves are decurrent not only in those types in which

they obviously appear to be so, but generally and probably universally throughout the group. This appears to be the case also in some at least of the *Pteridophyta*.

2. In those species in which a hypocotyl is developed, its superficial tissues are similarly derived from the cotyledons, and it is therefore to the cotyledons that we must look for an explanation of its external features.

3. This 'leaf-skin' is formed by a downward growth and extension of the leaf primordium, which keeps pace with the extension of the central axis with which it is fused, after the manner conceived by Hofmeister, who, however, in his *Berindung* theory goes so far (in the case of *Equisetum* specifically, and it would appear in the higher plants too) as to refer *all* the tissue external to the pith to the leaf.

4. The contact edges of these extensions may be so adjusted or so deeply fused as to show no outward trace. Or they may exhibit the characteristics of *potential* edges and be demarcated in various ways (by ridges, furrows, lines of hairs or of colour).

5. When each leaf-insertion completely encircles the stem (as in most Monocotyledons and a few Dicotyledons), or when the leaves, being opposite or whorled, come into lateral contact at the level of insertion (as in many Dicotyledons and some Conifers), the downward extension of each leaf is limited to a single internode.

6. Where the leaves are opposite, but where the insertion width occupies less than $\frac{1}{2}$ the circumference of the stem (especially well seen in some Dicotyledons with four-angled stems), the leaf-extension stretches through two internodes.

7. Where the leaves are spirally arranged with an insertion width less than the circumference of the stem, the pattern traced by the fused edges of the extensions and the number of internodes through which the extension of any one leaf descends will depend on the leaf-divergence and the leaf-insertion width.

8. If the leaf-divergence and the leaf-insertion width are expressed by the same fraction (e.g. $\frac{2}{5}$), the contact line descending from one edge of each insertion will 'pick up', and the one from the other will 'be picked up' by a corresponding line descending from the neighbouring leaf below and above respectively, and each fusion line thus formed will extend downwards until it terminates in the axil of a particular leaf in the cycle.

9. When the leaf-insertion width occupies some lesser fraction of the circumference no 'pick-up' occurs; the boundary lines run a separate course, and hence *two* terminate in each axil, one on either side the mid-point.

10. In the circumstances described under (8) and (9) the boundary line from one edge of the leaf-insertion extends through a greater number of internodes than the one from the other edge of the same insertion.

11. The length of axis traversed by the separate leaf-trace, though constant within narrow limits for the species, bears no direct relation to the limits of the leaf area laid down in the superficial tissue of the axis. Hence it may be said that the delimitation of an inner boundary to this region of the leaf is a *question solely of definition*, but that on the outer surface this boundary, in many types, has a *real existence capable of demonstration*.

12. Two forms of the surface pattern are met with, having the relation of mirror images, the individual or axis exhibiting the one or the other according as its general orientation is from right to left or from left to right.

13. If the leaf-insertion width is such that the insertions of all the leaves in one cycle of the spiral are together just equal to the circumference of the stem (e.g. $\frac{1}{5}$ in a $\frac{2}{5}$ spiral or $\frac{1}{8}$ in a $\frac{3}{8}$ spiral), the demarcations of the extensions will appear as continuous lines along the length of the axis.

14. In the case of flowering stems the leaf-skin is formed by the bracts (when present) and the outermost sepals.

15. The demarcation of leaf and cotyledon extensions by lines of hairs only occurs (so far as observed) when the basal free margins of leaves and cotyledons are furnished with hairs.

16. Demarcation of the boundaries of the leaf-extensions by lines of hairs is a character showing what may be termed *conditional* inheritance, in contradistinction to the type of inheritance which is (approximately) *constant* under all conditions.

17. A further fluctuating development of hairs in response to altered conditions may result in a general flooding of the surface, thus blurring the effect of the hereditary component. A similar secondary effect may be observed under appropriate conditions when the contours are traced out in anthocyanin.

18. The occurrence of adventitious exogenously formed buds on the hypocotyl (as e.g. in *Antirrhinum Orontium*) appears to show that a shoot can be formed directly from foliar tissue at undifferentiated points without previous mutilation or other injury.

19. Such buds may show a general flooding with anthocyanin in response to the different conditions existing in the embryonic tissue, while the parent axis has the colouring matter localized in a definite region.

In conclusion I wish to express my grateful thanks to Miss Margaret Willis, who made the drawings here reproduced. I have also to thank Herr W. Engelmann of Leipzig for his courtesy in allowing me to reproduce the two figures taken from Sachs's 'Lehrbuch' and the 'Vorlesungen'.

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On the Absorption of Ions by the Roots of Living Plants.

I. The Absorption of the Ions of Calcium Chloride by Pea and Maize.

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INTRODUCTION.

NUMEROUS observations are on record which suggest an unequal absorption of the ions of a single salt presented in solution to living plant cells. Thus, the long-recorded observation that water-culture solutions turn acid or alkaline after a time can be explained by supposing that the two ions of a neutral salt are absorbed to different extents, and that the excess of the more absorbed ion is accompanied in its entrance into the plant by an equivalent quantity of hydrogen or hydroxyl ion, according to whether the ion in question is negatively or positively charged, with the result that a corresponding quantity of hydroxyl or hydrogen ion is left in the external solution, which in consequence reacts alkaline or acid. Also Nathansohn (5, 6, 7), Meurer (4) and Ruhland (11), by immersing discs of plant tissue in various salt solutions of known concentration and analysing the solutions after a certain time, have obtained evidence of the unequal absorption of the two ions of the salts employed. The most extensive work on this problem is that of Pantanelli (8, 9, 10), who performed a long series of experiments with representatives of all the large groups of the Plant Kingdom.

He came to the conclusion that the ions of the salts were absorbed in unequal proportions. As a rule the anions were absorbed more rapidly than the kations, but several exceptions were recorded, notably sulphates with Dicotyledons, and chlorides with *Elodea*, where kations were absorbed more rapidly than anions. In the case of the marine algae *Ulva* and *Valonia* more calcium than chloride was absorbed from a solution of calcium chloride. Pantanelli also obtained evidence of a marked periodicity in absorption; at first the plants absorbed the salts rapidly, attaining

a maximum fairly early; this absorption was followed by a definite excretion of ions, and this was succeeded by a period of intake, so that the absorption-curve exhibited a series of maxima and minima. Pantanelli made a number of experiments to determine changes in the acidity or alkalinity of the culture solutions, and the results confirmed his former conclusions.

Johnson (3) observed a slightly unequal absorption of ions in a series of experiments with beet, carrot, and maize (white field corn and sweet corn) in calcium chloride solutions. He found that more calcium was absorbed in proportion by dead tissue than by living tissue in the case of beet, carrot, and sweet corn.

These results cannot be accepted without further confirmation, because neither Pantanelli nor Johnson took account of the magnitude of the biological error arising from individual differences in the plants examined, and they give no indication whether the differences they record are within or without the limits of this error.

How serious this source of error may be is well illustrated by some figures taken from the results of the present investigation.

In one case the absorption of chloride by one member of a group of pea plants was 5.64 per cent., and by another member only 2.878 per cent., while the average for the group was 3.578 per cent., with a probable error of 0.506.

A still more striking case of individual variation is taken from the results of a series of experiments with maize plants in which absorption of calcium by one individual was 5.291 per cent. and by another 30.23 per cent.; the average of the group was 17.53 per cent., with a probable error of 2.7.

Hoagland (2) questions the unequal absorption of ions from salts such as potassium chloride in solution; in his experiment the nutrient solutions remained neutral, or, if acid or alkaline to begin with, they gradually became neutral after contact with plant roots. Analysis of sodium nitrate solutions showed that more nitrate than sodium was absorbed; the bicarbonate was formed, so that the solution did not become alkaline. In the case of potassium chloride solutions the potassium and chloride were absorbed in equal proportions.

In the present research it was considered advisable to limit the investigation in the first place to the absorption of a single salt by plants of one or two species, and to obtain sufficient data to enable the probable error of experiment to be calculated, rather than to attempt experiments with a wide range of salts and species, in which it would be impossible to obtain sufficient data with any one salt or species to indicate the degree of accuracy of the results. The salt selected was calcium chloride, and the species used were the edible pea and maize.

A pure strain of seed of each species was obtained from Messrs. Sutton and Sons.

METHODS.

Seeds were germinated in sawdust watered with tap-water, and when the roots were two or three inches long the seedlings were transferred to a water-culture solution of the following composition (Crone (1)) :

KNO_3	1.0 grm.
$\text{Ca}(\text{NO}_3)_2$	0.25 grm.
MgSO_4	0.25 grm.
$\text{Fe}_3(\text{PO}_4)_2$	0.5 grm.
Distilled water up to 1,000 c.c.	

The plants were grown in this solution for about a fortnight in order to obtain healthy, uninjured roots. From these plants a number were selected which possessed, as far as could be judged, an approximately equal root development, and these were transferred to the calcium chloride solutions.

Three concentrations of the salt were employed :

$$\frac{\text{N}}{10}, \quad \frac{\text{N}}{100}, \quad \text{and} \quad \frac{\text{N}}{1000},$$

the solutions being contained in wide-mouthed bottles of 1,200 to 1,300 c.c. capacity, such as are generally used for water-culture experiments. Only one plant was grown in each bottle. The usual precautions necessary for growing plants in water-cultures were taken. After various times a number of plants were withdrawn from the experimental solutions. The following quantities were then determined in the solutions: (1) the concentration of calcium, (2) the concentration of chloride, (3) the electrical conductivity, (4) the hydrogen-ion concentration, (5) the presence of other metallic ions.

The calcium was determined gravimetrically as calcium oxide, and the chloride volumetrically. In the case of the more dilute solutions a large bulk of solution was evaporated down for each determination. As a number of plants were removed from each solution at the same time, it was possible to calculate the probable error of the results. The electrical conductivity was measured in the ordinary way by Kohlrausch's method, and the hydrogen-ion concentration was determined colorimetrically.

EXPERIMENTAL RESULTS.

Series 1. A number of plants of the edible pea were grown in the culture solution indicated in the preceding section, and groups of sixteen plants transferred to each of the three calcium chloride solutions :

$$\frac{\text{N}}{10}, \quad \frac{\text{N}}{100}, \quad \frac{\text{N}}{1000}.$$

Four plants from each group were removed from the culture solutions after a lapse of 36 hours, another four from each group after 60 hours, and the remaining four of each group after 84 hours. The mean percentage absorption of calcium and chloride in each case is set out in the two following tables along with the probable error of the mean, though, owing to the small number of plants used, the errors can claim no high degree of accuracy.

TABLE I.

Percentage absorption of calcium by roots of edible pea from various strengths of calcium chloride solutions.

Concentration.	36 hours.	Percentage absorption after 60 hours.	84 hours.
$\frac{N}{10}$	17.74 $\pm$ 1.376	12.77 $\pm$ 1.84	14.61 $\pm$ 1.68
$\frac{N}{100}$	19.61 $\pm$ 2.33	8.244 $\pm$ 1.471	—
$\frac{N}{1000}$	23.10 $\pm$ 5.30	—	—

TABLE II.

Percentage absorption of chloride by roots of edible pea from solutions of calcium chloride of various strengths.

Concentration.	36 hours.	Percentage absorption after 60 hours.	84 hours.
$\frac{N}{10}$	3.578 $\pm$ 0.506	4.021 $\pm$ 0.073	3.856 $\pm$ 0.351
$\frac{N}{100}$	12.47 $\pm$ 1.66	10.66 $\pm$ 0.856	12.47 $\pm$ 1.16
$\frac{N}{1000}$	15.09 $\pm$ 3.736	—	—

In the case of solutions of $\frac{N}{1000}$ concentration, the results became very irregular after the first day, and the analyses were not continued. With a concentration of $\frac{N}{100}$ the results of the calcium analyses were irregular after the second day, so that no average for the absorption of calcium could be calculated for the third day. On the fourth day there was no uniformity in the results obtained from any of the analyses, so no averages were calculated.

The mean electrical conductivity of the solutions for each day is given in Table III.

TABLE III.

Relative electrical conductivity of solutions of calcium chloride in which peas had been growing for different lengths of time.

Concentration.	Relative electrical conductivity after		
	36 hours.	60 hours.	84 hours.
$\frac{N}{10}$	0.05225	0.053	0.0524
$\frac{N}{100}$	0.00669	0.00716	0.00722
$\frac{N}{1000}$	0.000707	0.000745	—

Series 2. A second series of experiments with peas confirmed the unequal absorption of ions from the solutions, by the roots of those plants, which was shown by the results of the first series.

From these experiments it is clear that a concentration of calcium chloride approximately $\frac{N}{10}$ is the most satisfactory for experimental purposes, as the results with this concentration throughout were more uniform than those obtained with the more dilute solutions; further, toxic effects were apparently postponed until the plant had been growing in the solution for three or four days, whereas, with the more dilute solutions, the harmful effects, indicated by the irregularity of the results, appeared before sixty hours had elapsed after the cultures were started.

Accordingly, in later experiments the plants were all grown in calcium chloride solutions of approximately $\frac{N}{10}$ concentration.

Series 3. Maize plants which had been growing in tap-water for about a fortnight were used for these experiments; the plants were divided into three sets, each set consisting of six plants, and the plants were removed after they had been growing in the experimental solutions for 23, 48, and 57 hours respectively. A large percentage absorption was obtained in every case; approximately the same amount of chloride was absorbed by each set, so that equilibrium is evidently reached very quickly; in the case of calcium, equilibrium is reached before 48 hours have elapsed. The results are given in Table IV.

TABLE IV.

Percentage absorption of calcium and chloride by roots of Zea Mais from $\frac{N}{10}$ solutions of calcium chloride.

Duration of experiment.	Percentage absorption of calcium.	Percentage absorption of chloride.
23 hours	17.53 $\pm$ 2.7	6.512 $\pm$ 0.1227
48 hours	25.28 $\pm$ 1.04	6.54 $\pm$ 0.2202
57 hours	26.98 $\pm$ 2.2	5.875 $\pm$ 0.2597

The hydrogen-ion concentration was determined by the colorimetric method for all solutions in which plants of *Zea Mais* had been growing. With two exceptions, the P_H of the solutions remained 7.3, the same as that of the original solution; in the two exceptional cases the solutions were slightly more alkaline, having a P_H of approximately 8.0.

The fact that the solutions remained approximately neutral, in spite of the more rapid absorption of calcium than chloride, suggested that other metallic ions might have diffused out from the roots, to replace the calcium. Accordingly, qualitative tests were made, and it was found that potassium and magnesium were present in appreciable quantity in the external solutions. The replacement of part of the excess of calcium ions absorbed, by the much more mobile potassium ions, might be sufficient to account for the slight increase in electrical conductivity of the external solution recorded in Table III.

DISCUSSION.

(a) *Unequal absorption of ions.* The results given above show that the two ions of calcium chloride are absorbed at different rates by the roots of young plants of pea and maize. The percentage absorption of calcium is considerably in excess of the percentage absorption of chloride throughout the experiments. Thus the conclusion reached by Pantanelli, as regards the unequal absorption of ions, is confirmed by these results. It is noteworthy that the kation is absorbed more rapidly than the anion throughout the experiments. Pantanelli's results show a higher absorption of the anion, except in the case of sulphates, and in experiments with *Elodea*; the latter absorbed kations more rapidly than anions when chlorides were used. Pantanelli, however, did not use peas, though he used closely related species in his experiments (*Phaseolus multiflorus*, *Vicia faba*, *Lupinus albus*).

The difference in the rate of absorption of the two ions is very much lessened in more dilute solutions, and when the concentration is as low as $\frac{N}{1000}$ this difference is hardly outside the range of experimental error. In this case the results fall into line with those obtained by Hoagland (2).

The results of the experiments on the hydrogen-ion concentration of the solutions in which maize plants had been grown show that the P_H remains approximately the same as that of the original solution ($P_H = 7.3$) in spite of the more rapid absorption of calcium than chloride. Hence the excess of calcium absorbed is not accompanied by hydroxyl ions in its entrance into the roots. Further experiments show that potassium and magnesium diffused out from the roots, and presumably they take the place, in the external solution, of the excess of calcium absorbed.

(b) *Course of absorption.* The results obtained in these experiments seem, at first sight, to indicate a slight periodicity in absorption, as described

by Pantanelli; consideration of the probable error in the present case, however, shows that no real significance can be attached to the differences. The average percentage absorption for each day is approximately equal in the case of peas, having regard to the magnitude of the probable error. In the case of maize there is a distinct rise in the percentage absorption of calcium on the second day. This indicates that equilibrium is reached quickly, probably during the first twenty-four hours that the plant is growing in the experimental solutions, with peas, but rather later with maize.

The fall in absorption at the end of the experiment is probably due to the toxic effect resulting from the presence of an unbalanced solution external to the roots.

It is hoped to extend these observations to other species and solutions and also to mixed solutions.

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SUMMARY.

1. Further evidence has been obtained in support of the unequal absorption of the ions of a salt by the roots of growing plants.

2. The difference in the rate of absorption of the ions was greatly reduced in less concentrated solutions; in very dilute solutions, which alone affect the plant under normal conditions, it was almost nil.

3. No evidence in support of periodicity in absorption was obtained.

4. Equilibrium in the intake of this single salt, by roots of living plants, is reached within the first twenty-four hours of exposure to the salt solution in the case of peas, and within the first forty-eight hours with maize.

5. Potassium and magnesium diffuse out from the roots to replace the excess of calcium absorbed, which explains how it is that the hydrogen-ion concentration of the solutions remained approximately the same.

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On Hybridization of some Species of *Salix*. II.

BY

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I. SUMMARY OF EARLIER RESULTS.

FIRST I will briefly recapitulate here what I have stated in my former paper concerning the same subject,¹ in so far as it is related to the present communication, because this will conduce much to clearness.

The hybridizations between two Japanese species of *Salix*, viz. *gracilistyla* and *multinervis*, were done in 1910 and 1911. The character which sharply distinguishes them is among others the hairy condition of their catkin: that of the former is characterized by being densely covered with greyish hairs, whilst that of the latter is very sparingly hairy (cf. Ikeno, l. c., Pl. I, Figs. 1–2). The cross *multinervis* × *gracilistyla* done in 1910 has given rise to fourteen F_1 hybrids. The latter were not uniform: eleven were provided with catkins densely hairy like *gracilistyla* (hence called *plants of G-type*), and three with catkins sparingly hairy like *multinervis* (hence called *plants of M-type*). It is to be noticed that each of the two parents was examined in respect to its genetical purity by means of fertilization *inter se*, and found to breed true to its respective type. The fertilization between male and female G-type has given rise in F_2 to many G-types and a few M-types, and that between male and female M-type to many M-types and a few G-types, while that between male G-type and female M-type has produced both types in almost equal number. I have tried to explain the formation of two types of progeny in F_1 , despite the apparent genetical purity of both parents, by the action of invisible factors.

¹ Journ. of Genetics, 1918, vol. viii, pp. 35–58.

The result of the same hybridization done in 1911 was quite different from that of 1910. It has given rise to almost fifty plants which were not hybrids, as was expected, but *multinervis*, i. e. identical with the mother-plant; all were found to be female.

II. FORMATION OF HYBRIDS.

1. General and F_1 Generation.

Though, as stated above, *multinervis* $\times$ *gracilistyla* has always given rise to a certain number of progeny, its reciprocal cross, i. e. *gracilistyla* $\times$ *multinervis*, was unsuccessful even by repeated trials (done in 1912 and 1918), and I stated in my former paper that this hybridization is wellnigh impossible.¹ This conclusion was, however, rather hasty. In 1919 I once more tried this operation, and was much astonished to see that this time I obtained success, though scanty. I have pollinated many ears, but only three have given some seedlings and I have obtained twenty-one hybrids in all. Of these four have died down, and I have now seventeen planted in my garden; they have not flowered yet, except two, each of which has borne G-type female catkins. So far as I am able to judge by their leaves, the remaining fifteen are certainly hybrids exactly similar to those ex *multinervis* $\times$ *gracilistyla*. The definite classification of all of them according to the nature of their catkins is thus still impossible, and they are consequently of not much use in my present experiment. I wish merely to point out the fact that *gracilistyla* $\times$ *multinervis* may be successful, though very rarely.

From *multinervis* $\times$ *gracilistyla* carried on in 1910 I got only fourteen hybrids. As this number is very small I repeated the same hybridization in 1918, and obtained many more offspring. Though seven of them have not yet produced flowers, those with flowers may be classified as follows:

TABLE I.

No. of Cross.	Progeny.					No. of Cross.	Progeny.				
	G-type.	M-type.	GM.	MM.	Totals.		G-type.	M-type.	GM.	MM.	Totals.
1	1	...	...	...	1	16	1	...	...	...	1
2	5	...	...	...	5	17	...	1	...	...	1
4	1	1	...	...	2	18	1	...	...	...	1
5	1	...	...	...	1	19	1	...	...	...	1
6	1	1	...	...	2	20	2	...	...	...	2
7	8	...	...	...	8	22	1	...	...	...	1
8	4	...	...	...	4	23	1	...	...	...	1
10	3	2	...	...	5	26	4	...	...	1	5
11	14	...	1	...	15	28	4	3	...	...	7
13	5	1	...	...	6	29	...	...	...	1	1
14	...	1	...	...	1	30	1	1	...	...	2
						Totals	59	11	1	2	73

¹ l. c., p. 36.

ERRATA

PAGE 176, Table I, for the sixth line of figures, 7 8 8 read 7 2 2. Also below the eleventh line of figures on the left half of the table, a new line of figures, 15 6 6, should be inserted.

Annals of Botany, Vol. XXVI, No. CXLII. April, 1922.

Each number in the above table includes plants derived from one catkin, and all catkins are taken from one tree. The table shows that very few plants are produced from each catkin—very often only one. This is first of all to be ascribed to the fact that each catkin is very poorly fertile, owing evidently to the great difficulty of the hybridization under discussion. Many more seeds were actually obtained than might be inferred from a simple inspection of the table; many of them, however, failed to germinate, or else many seedlings perished in very young stages. In the table Nos. 3, 9, 12, 21, 24, 25, and 27 are wanting, because no adult plants were available from these pedigree numbers, owing to one of the two causes stated above. In one plant from No. 11, ranked under the column GM, some branches bear G-type catkins, and others M-typed ones. Nos. 26 and 29 have each produced one female *multinervis* (placed under the column MM), to which I will refer later in this paper.

If we add together the F_1 hybrids from the crosses done in 1910 and 1918 we have:

TABLE II.

Year.	G-type.	M-type.	Multinervis.	GM.	Totals.
1910	11	3	...	...	14
1918	59	11	2	1	73
Totals	70	14	2	1	87
%	83.3	16.7	...	...	...

If we take simple G- and M-types into consideration, the three remaining plants being left out of account, we have eighty-four offspring in all, composed of 83.3 per cent. G-types and 16.7 per cent. M-types.

The important question in respect to the hybridization under discussion is: Why will it yield the two types G and M already in F_1 , despite the fact actually observed that each of the two parents breeds true respectively? Have we here to deal with a segregation? In my former paper I was led to the conclusion that we have in this case no segregation at all, and I tried to explain the appearance of two types in F_1 , as well as the behaviour of the F_2 progeny, by the interference of invisible factors X and Y .¹ The results of hybridizations between F_1 hybrids and either of the two parents (i. e. back-crossing) which were performed in 1917 and 1918 served for testing the validity of my hypothesis. They were by no means particularly favourable for it. Especially, the results from G-type $F_1 \times gracilistyla$ were contrary to my theoretical expectation: thus, if we denote by X the factor which is able to cause Gg to develop to G-type, as was done in my former paper,² we have G-type $F_1 = XXGg$, and *gracilistyla* = GG , and the cross $XXGg \times GG$ or $XXGg \times xxGG$ should give rise to $2(XxGG + XxGg)$, i. e. to G-type progeny exclusively, which was not

¹ l. c., p. 48 ff.

² l. c., p. 50 ff.

actually the case, inasmuch as I have got by this crossing not only G-type, but a certain number of M-types (see Table V, Expt. 6, below). Such a fact, as well as certain other reasons which would not be needed here to be enumerated, have led me to abandon my hypothesis, at least in the form in which it was expressed in my former paper. How can we then explain the phenomenon? For the reasons which I will not repeat here I was formerly unwilling to accept the 'imperfection of dominance' for its explanation.¹ But if the view of 'invisible factors' is to be abandoned, I think that there will be no better means than to resort to that of the 'imperfection of dominance', for the latter will explain most simply what we observe in respect to *Salix* hybrids; though I am still of the opinion that the interference of certain other factors, either visible or invisible and variously combined, may be the ultimate cause of this phenomenon. What is discussed below is consequently founded on the view of the 'imperfection of dominance'.

We have seen in Table I that in F_1 of *multinervis* $\times$ *gracilistyla* we have 83.3 per cent. G-types and 16.7 per cent. M-types. We may regard the densely hairy condition of the catkin of *gracilistyla* to be dominant to the less hairy condition of that of *multinervis* as a rule, so that if we represent the former by *D* and the latter by *R*, all F_1 hybrids will agree in being *DR* genetically. The dominance here is not absolute; consequently only in the 83.3 per cent. is the dominant character observed, whilst in the remaining 16.7 per cent. it fails, and lets the recessive character appear externally. In short, the G-type as well as the M-type progeny in F_1 are *genotypically* equal, though *phaenotypically* different; we have evidently no segregation in this generation. Moreover, Table I shows us that many numbers therein, each of which corresponds to one catkin, contain both G- and M-types. Hence we may conclude that not only one plant individual, but also even one and the same catkin, possesses at the same time both gametes where usual dominance of *D* over *R* prevails and those where it fails.

2. F_2 Generation.

In order to obtain the F_2 progeny I have carried on the three following crosses between the G-type and M-type progeny.² The results are shown in the following table³:

¹ l. c., p. 48.

² Only in Expt. 3 is the male parent derived from F_2 (l. c., p. 42).

³ The number of progeny in Expts. 1 and 2 is the same as in my former paper (l. c., pp. 42-3), but that in Expt. 3 is a little larger, because I have added afterwards those plants which have borne flowers in 1919 and 1920. In calculating the percentage the new type was not taken into account.

TABLE III.

No. and Nature of Experiment.	Progeny.			Totals.
	G-type.	M-type.	New type.	
1. (G-type ♀ × G-type ♂)	187	32	...	219
2. (M-type ♀ × G-type ♂)	77	67	15	159
3. (M-type ♀ × M-type ♂)	4	19	8	31
Totals	268	118	23	409
%	69.4	30.6	...	...
Deviation from 75% : 25% expectation	-5.6	+5.6	...	...

Plants ranked under the column 'New type' in the above table have their catkins very similar to those of M-type, but distinguished from the latter by 'their perfect non-hairiness (l. c., p. 44). In my former paper I stated my supposition that they will perhaps breed true in later generations. In order to confirm it many seeds of this type produced by fertilization *inter se* were sown in 1918, and many germinated, but a large portion of the seedlings perished in the young stage, and I obtained only eleven adult plants. Of the latter two produced flowers in 1920, and proved themselves to be the male and female plant respectively, and are quite similar to their parents externally. The remaining nine do not bear flowers yet. My supposition was thus confirmed, at least partially. The problem, whether they were produced by the so-called 'loss-mutation' or by the new combination of certain factors, would not be definitely decided without further breeding experiments.

Since the crosses shown in Table III correspond to $DR \times DR$, we expect to get 75 per cent. D and 25 per cent. R , if we are dealing here with the 3 : 1 segregation. We have actually 69.4 per cent. G-types and 30.6 per cent. M-types, i. e. ± 5.6 per cent. deviation from this expectation. The deviation is *positive* on the side of M-type progeny, and this fact is evidently due to the imperfection of dominance: the F_2 progeny are to be represented genotypically by 25 per cent. DD + 50 per cent. DR + 25 per cent. RR ; in the case under discussion in 5.6 per cent. out of 50 per cent. DR the dominance failed, and the recessive character has made its appearance, or, in other words:

25 per cent. DD + 44.4 per cent. DR + 5.6 per cent. (DR) + 25 per cent. RR = (25 + 44.4) per cent. G-type + (5.6 + 25) per cent. M-type = 69.4 per cent. G-type + 30.6 per cent. M-type.

(DR) denotes the zygotes where the usual dominance fails.

In short, the segregation follows the 3 : 1 mode, and the deviation is due to the imperfection of dominance of the factor D over R .

3. Back-crosses.

The results of back-crossing of F_1 hybrids by either of the two parents, i. e. $DR \times R$ or $DR \times D$, have fully confirmed those obtained in the F_1 as well as the F_2 generation.

In respect to the back-cross $DR \times R$ we have to distinguish between two cases, viz. G-type $\times$ *multinervis* and M-type $\times$ *multinervis*. They were carried on in 1918, and the results as studied in 1920 and 1921 are as follows:

TABLE IV.

No. and Nature of Experiment.	Progeny.		
	G-type.	M-type.	Totals.
4. (G-type ♀ × <i>multinervis</i> ♂)	125	88	213
5. (M-type ♀ × „ „)	14	91	105
Totals	139	179	318
%	43.7	56.3	...
Deviation from 50 % : 50 % expectation	-6.3	+6.3	...

Since the segregation occurs according to 3:1 fashion, we expect to have in $DR \times R$ 50 per cent. G-types and 50 per cent. M-types. Actually we have 43.7 per cent. of the former and 56.3 per cent. of the latter. The deviation is therefore ± 6.3 , and is *positive* on the side of the M-type, just as was the case in F_2 (see Table III); this is evidently to be ascribed to the fact that in certain cases the usual dominance fails, i. e.—

$DR \times RR = (50 - 6.3)$ per cent. $DR + 6.3$ per cent. $(DR) + 50$ per cent.
 $RR = 43.7$ per cent. G-type + $(6.3 + 50)$ per cent. M-type = 43.7 per cent.
 G-type + 56.3 per cent. M-type.

(DR) denotes, as before, the zygotes where dominance fails.

In the back-cross $DR \times D$ we have to distinguish between G-type $\times$ *gracilistyla* and M-type $\times$ *gracilistyla*. These crosses were performed in 1919, and the results as studied in 1921 are as follows:

TABLE V.

No. and Nature of Experiment.	Progeny.			
	G-type.	M-type.	New type.	Totals.
6. (G-type ♀ x <i>gracilistyla</i> ♂) . . .	184	8	...	192
7. (M-type ♀ x " ") . . .	76	9	11	96
Totals	260	17	11	288
%	93.8	6.2	...	...
Deviation from 100 % : o/o expectation	-6.2	+6.2	...	...

In the above table the new type is not taken into consideration for counting the percentage.

Since the above crosses correspond to $DR \times D$ we expect to have 100 per cent. G-types and no M-types at all. Actually we have 6.2 per cent. of the latter, so that the deviation from the theoretical expectation is ± 6.2 , which is again *positive* on the side of M-type, i. e.—

$DR \times DD = 50$ per cent. $DD + (50 - 6.2)$ per cent. $DR + 6.2$ per cent.
(DR) = 93.8 per cent. G-type + 6.2 per cent. M-type.

(DR) = zygotes where dominance fails.

If we compare the results shown in Tables III, IV, and V, we see that the deviations from the expectation are very similar to each other (5.6 per cent., 6.3 per cent., 6.2 per cent. respectively), which will indicate that the degree of failure of dominance is nearly the same in all three cases. In F_1 generation, however, where the theoretical expectation is 100 per cent. dominants and no recessives, the deviation, i.e. the failure of dominance, is 16.7 per cent. (see Table II), and consequently much larger than in the above three cases. To what cause may such a difference be ascribed? We may make several suppositions. Thus, for instance, in the crosses shown in Tables III–V one or both parents are heterozygous, i.e. DR , whilst in the cross in Table II both are homozygous; may not such a circumstance lead to the difference in the degree of failure of dominance? We may perhaps put forward another consideration: Does not that degree vary under the influence of external conditions? Such questions and all others which one may ask are naturally not to be definitely decided without further breeding experiments, and I must here be satisfied with simply indicating such problems.

A few words as to the dominance of the *gracilistyla* catkin. We have seen that the hairy condition of catkins of the latter is dominant to the less hairy condition of *multinervis* as a rule. I have observed that in *multinervis* $\times$ *gracilistyla* the non-hairiness of leaves is dominant to their hairiness (l. c., p. 38), which is consequently very different from what we see in the catkins in the same hybrid. Evidently the genetic factors concerning hairs covering leaves on one hand and those covering catkins on the other must be quite different.

4. Potency.

As we can easily see by comparing the results of the various crosses above enunciated, the degree of failure of dominance is not the same in G-type as in M-type. The question is, Do the G-type plants produce a larger proportion of G-type progeny than the M-type ones, and vice versa? Is the degree of 'potency' (to use the word and the expression adopted by Davenport¹) inherited?

Let us first see what other authors have observed in this respect. In their experiment on the crossing between extra toes and normal ones in poultry, where the former are dominant to the latter, Bateson and

¹ Carnegie Institution of Washington Publication, No. 121, 1909. The author says (p. 92), 'The potency of a character may be defined as the capacity of its germinal determiner to complete its entire ontogeny. If we think of every character as being represented in the germ by a determiner, then we must recognize the fact that this determiner may sometimes develop fully, sometimes imperfectly, and sometimes not at all. . . . Potency is variable. Even in a pure strain a determiner does not always develop fully. . . . But in a heterozygote potency is usually more or less reduced. When the reduction is slight, dominance is nearly complete; but when the reduction is great, dominance is more or less incomplete. . . .'

Punnett¹ have obtained, (a) from the heterozygote *DR* with usual dominance $\times$ recessive, 114 extra toes and 95 normal, i. e. 54.5 per cent. and 45.5 per cent. respectively, and (b) from the heterozygote *DR* where dominance fails $\times$ recessive, 128 extra toes and 151 normal, i. e. 45.7 per cent. and 54.1 per cent. respectively; consequently the class (a) contains comparatively a little more extra-toed progeny than the class (b). So that there may be a very slight inheritance of degree of potency, but this is quite insignificant. Much more marked inheritance has been found by Davenport² in respect to the crosses of Houdan fowls: he has calculated the correlation between total number of toes in the two parents and average number of toes in their progeny, and found the coefficient of correlation to be equal to $+0.683 \pm 0.092$.

Turning to our case, the simple inspection of the results of Expts. 1, 3, 5, 6 shown in Tables III, IV, V, will immediately indicate to us that the inheritance of the degree of potency is very significant in the present case. The results of our four experiments just enumerated were employed to make the following table of correlation:

TABLE VI.

Nature of Crosses.	Progeny.		Totals.
	G-type.	M-type.	
G-type $\times$ G-type and G-type $\times$ <i>gracilistyla</i> (Expts. 1 and 6)	187 + 184	32 + 8	411
M-type $\times$ M-type and M-type $\times$ <i>multinervis</i> (Expts. 3 and 5)	4 + 14	19 + 91	128
Totals	389	150	539

This gives $r = +0.724 \pm 0.021$. The coefficient of correlation is rather high, and this result confirms our supposition founded on mere inspection of data.

Since the appearance of the well-known work of Wichura³ it seems that botanists have generally believed in the constancy of *Salix* hybrids throughout later generations. In a short note Sirks⁴ has expressed his opinion that the work of Wichura by no means induces us to the belief that *Salix* hybrids are constant. In 1916⁵ and 1918⁶ I published the results of my investigations on the hybridization of some species of *Salix*, especially *S. gracilistyla* and *multinervis*: in these experiments I was able, contrary to the current belief of botanists, to observe the segregation of some few characters in *Salix* hybrids, but since the relative number

¹ Reports to the Evolution Committee of the Royal Society. Report II, 1905, p. 115.

² l. c., p. 23.

³ Die Bastardbefruchtung im Pflanzenreich erläutert an den Bastarden der Weiden. Breslau, 1865.

⁴ Zeits. f. indukt. Abstamm.- u. Vererbungslehre, Bd. xv, 1915, pp. 164-5.

⁵ Bot. Mag., Tôkyô, vol. xxx, 1916, pp. 316-20.

⁶ Journ. of Genetics, vol. viii, 1918, pp. 35-58.

of segregates was somewhat different from what we might have expected in the case of usual Mendelian segregation, I could not then decide definitely whether we have here really to deal with such or not. In 1918 Heribert-Nilsson¹ published an elaborate memoir containing the results of his extensive experiments on *Salix* hybrids: he was able not only to prove the segregation of various characters, but also to discover the usual Mendelian ratio in many cases. My experiments concerning the catkin characters above mentioned have shown that their segregation occurs according to the simplest Mendelian ratio 3 : 1, though more or less obscured by the imperfection of dominance. Accordingly these experiments are a further contribution towards establishing the fact that the segregation of the various characters in *Salix* hybrids follows Mendel's law.

III. FORMATION OF PARENTAL FORM.

The hybridization *multinervis* × *gracilistyla* done in 1911 has given rise to almost fifty offspring, all of which were, contrary to our expectation, not hybrids, but female *multinervis*.²

I have myself had no doubt about the actual production of *multinervis* progeny in this case. The fact was, however, so unexpected that others may reasonably be in doubt about its reality. So I repeated the same hybridization in 1918 and 1919. The results of hybridization in 1918 are contained in Table I (see p. 176). Though, as is usually the case in this hybridization, each catkin has produced only few seeds—often none—yet I have obtained a comparatively large number of progeny—more than ninety plants in all; but the number of *multinervis* was small—in fact I had only two (Table I, Nos. 26 and 29). On the contrary, the hybridization carried on in 1919 has yielded very few seedlings, but almost all were *multinervis* progeny, thus:

TABLE VII.

No. of the Female Parent (<i>multinervis</i> ♀).	No. of Catkins.	No. of Progeny (<i>multinervis</i>).
I.	35	2
	36	1
	41	1
II.	4	1
	7	4
Total		9

Gracilistyla used as the pollen plant in the above experiments was the same in both cases, and identical with what we have employed in the experiments whose results are shown in Table I.

No progeny contained in the above table bear flowers yet, but that all of them are *multinervis* is quite unmistakable on account of their characteristic leaves, &c. Besides them, some plants which are not *multi-*

¹ Lunds Universitets Årsskrift, N.F., Afd. 2, Bd. xiv, No. 28, 1918.

² l. c., p. 51.

nervis, perhaps hybrids, have been produced, but since they perished before flowering they are not enumerated in the table.

From the results just enunciated we now can have no reason to doubt the production of *multinervis* progeny from *multinervis* mother without the action of *multinervis* pollen.

It is well known that sometimes in some *Salix* species a few male flowers are produced on the female catkin, or even a few flowers become hermaphrodite. One might perhaps think that the production of *multinervis* progeny in our case might be due to such abnormalities which have escaped my attention. I have looked for them in our *multinervis* trees carefully and repeatedly: it may be remarked in this respect that I have never met with even traces of such in our trees.

When my former paper was published the fact whether or no the *multinervis* progeny under discussion will breed true in later generations was not yet decided, though this seems to me to be highly probable. I pollinated in 1920 two such *multinervis* progeny (from Nos. 26 and 29 in Table I) by pollen taken from a *multinervis* male plant, and got a certain number of progeny. The latter were *multinervis* without exception, so that my supposition that they will breed true has been fully confirmed.

The next question will be, How are the *multinervis* progeny produced without the action of *multinervis* pollen? My conclusion in my former paper was that it may be ascribed neither to parthenogenesis nor to development of nucellar cells, but to so-called pseudogamous development of oospheres due to the stimulus of foreign pollen (l.c., pp. 51-4). Some doubt, however, arose in my mind in 1919 about this conclusion. Mr. S. Nohara, then assistant in my laboratory, covered a number of young catkins of a tree cultivated in our botanical garden, and very similar to *multinervis*,¹ with paper-bags for a certain purpose, and left them in this condition for a certain lapse of time, perhaps about two months. On opening them he was struck by the fact that some catkins had produced a few fruits. He notified me of the fact. I placed some seeds thus obtained upon a moist filter-paper within a Petri dish: some of them were so weak that they refused to germinate, but others germinated very well. Still others were sown in a seed-pan and came to germination.² From all these facts there will be no doubt about the occurrence of the 'apomictic' development without any pollination in this case, to use the word introduced by the German botanist Winkler.³

¹ It may be a variety of *multinervis*; it is chiefly distinguished from the latter by its smaller catkins.

² Plants derived from this germination are now cultivated in our garden. So far as we can judge by their leaves they are quite similar to their parent; one of them has already borne female catkins, and proved itself to be perfectly similar to its parent.

³ Parthenogenesis und Apogamie im Pflanzenreiche, Jena, 1908, pp. 8 ff.

In my former paper I stated the fact that female inflorescences of *multinervis* covered with paper-bags were never able to bear fruits.¹ This is quite true, but then only a few branches were used for the experiment. In view of the results obtained by Mr. Nohara, which are described above, the experiments were repeated in 1920 and 1921 on a much larger scale than formerly. In 1920 nine trees were chosen for the experiment: of these, three (Nos. 1-3 in Table VIII below) which are high and copiously branched are derived from the fertilization *inter se* of male and female trees used in my experiments done in 1910 and 1911; the remaining six are smaller, of which four (Nos. 4-7 in Table VIII) are the cuttings from a female tree used in the same experiments, and two are from Nos. 26 and 29 in Table I (i.e. plants produced without the action of *multinervis* pollen), designated in Table VIII as Nos. 8 and 9. The number of catkins in each tree was as follows:

TABLE VIII.

No. of Trees.	No. of Catkins.
1	1,105
2	326
3	818
4	20
5	29
6	19
7	36
8	55
9	95
Total	2,503

If we suppose that each catkin bears 100 flowers on average, and each flower contains three ovules, both of which are by no means high estimations, we should have a total of 750,900 ovules. All catkins enumerated in the above table were covered with paper-bags, and left in this condition for nearly two months. Though in certain catkins a few ovaries grew up somewhat more intensely than others, and consequently attracted our attention by their remarkable size, yet all of them, on opening, were found either to be quite empty or to contain a few seed-hairs only. All other catkins simply dried up and fell, with the exception of two catkins from No. 8 in the above table. Thus only from the latter did I get four seeds in all. Of these, two, being placed upon a moist filter-paper within a Petri dish, came to germination, whilst the remaining two did not.

Similar experiments were repeated in 1921 on the same nine trees used in 1920. The number of catkins produced in 1921 was somewhat smaller than in 1920; besides, either a certain number of branches covered with bags were broken down, or the latter were torn off by storms which

¹ l. c., p. 54.

raged several times in March and April. As catkins on such branches must necessarily have been left out of consideration, the number of those available for our experiment was considerably diminished. Nevertheless, we had 1,261 catkins which should contain no less than $1,261 \times 100 \times 3 = 378,300$ ovules in all, i.e. more than half the number of ovules experimented upon in 1920. All these catkins were covered with bags for more than two months. As in the former year I saw not unfrequently ovaries which grew somewhat more intensely than others, but generally they either simply shrivelled up gradually, or opened and proved themselves to contain no seeds at all. Only on some catkins of the tree designated as No. 1 in Table VIII did I get eight seeds. Of these, three came to germination, and one began to germinate but soon ceased to make any further growth. All remaining seeds refused to germinate.

In the two new experiments just mentioned I was thus able to get only very few seeds which have the power of germination, but nevertheless they have proven beyond all doubts that in our case the apomictic development of ovules without the application of any pollen is a matter of possibility, though it is of extraordinarily rare occurrence.

In consequence of the results of the new experiments just described my former view concerning the development of *multinervis* after the pollination with *gracilistyla*, as stated in my former paper, must necessarily change. To explain the result of our new experiments there are two possibilities, viz. the embryo formation from nucellar cells and the parthenogenesis, each of which is either autonomous or induced by the stimulus of foreign pollen. The definite conclusion whether the apomictic development in question will occur according to the first or the second mode just enunciated would naturally be possible only after a comparative cytological examination of the development of normal as well as apomictic ovules. Since, as above stated, ovules of the latter class are of extremely rare occurrence, it would be necessary for the purpose to examine innumerable specimens made by sectioning an enormous number of catkins, and even then there would be no great chance of meeting with such ovules. Thus we see that in the present case the cytological examination must be almost an impossibility. The embryo formation from nucellar cells is generally accompanied by polyembryony, as in *Funkia*, *Citrus*, *Opuntia*, &c., &c. Since I have never met with this in *Salix*, although I have observed the germination of many thousand seeds of various species of *Salix*, either pure or hybrid, including *S. multinervis*, I am rather inclined to the view that as the cause of apomictic development in our case parthenogenesis is much more probable than embryony. All the following discussion is accordingly founded on the supposition that we have here to deal with parthogenesis, either autonomous or induced.

In *Thalictrum purpurascens*¹ as well as some species of *Hieracium* (*pilosella*, *excellens*, &c.)² it is well known that there are two kinds of ovules, viz. those which require fertilization for seed formation, and those which do not. It will naturally be quite the same in *Salix multinervis*. As we have here to deal in all probability with somatic parthenogenesis in the sense of Winkler,³ it follows that the former kind of ovules, which have undergone the reducing division of chromosomes during their development, become hybrids by pollination with foreign pollen, while the latter kind, which have undergone no such process, develop to *multinervis* without being fertilized. Moreover, that these two kinds of ovules are contained at the same time not only in one plant individual, but also in one and the same catkin, is clearly seen from the examination of No. 26 in Table I (see p. 176), inasmuch as in this number the progeny which are derived from one single catkin contain, besides one *multinervis*, four G-types (i. e. hybrids).

In my former paper I was led to the conclusion that in our case we have to deal with pseudogamy, i. e. parthenogenesis induced by the stimulus of foreign pollen, *gracilistyla* in the present case. This was the natural consequence deduced from the results of my former experiments, because then any catkin covered with a bag was found not to be able to bear a single seed. Since, however, our new experiments have shown us that in certain cases, though extremely rare, catkins wholly prevented from any pollination are able to produce a few seeds, my former conclusion is of course not quite right. The following remarks may, however, be made in consequence of the results of our new experiments. Although very rarely, the apomictic development without any pollination is possible, yet we have seen that the number of seeds then produced was so scanty that pseudogamy might also be not impossible in certain cases. For instance, in our experiment done in 1911, where the pollination with *gracilistyla* was several times carried on, I have got almost fifty *multinervis* individuals from a few catkins, while in the experiments done in 1920 and 1921, when no such pollination was practised, only five apomictic seeds which were able to germinate were obtained out of more than $700,000 + 370,000 = 1,070,000$ ovules!⁴ It is not improbable that although few ovules may develop parthenogenetically without the stimulus of foreign pollen (autonomous parthenogenesis), yet ovules may in many cases be induced to

¹ Overton: Bot. Gaz., vol. xxiii, 1902, pp. 363-75; Ber. d. Deutsch. Bot. Ges., Bd. xxii, 1904, pp. 274-83.

² Ostenfeld: Botanisk Tidsskrift, Bd. 27, 1906, pp. 225-48. Rosenberg: ibid., Bd. xxvii, 1907, pp. 143-70.

³ l. c., p. 17.

⁴ In the experiment carried on in 1919, when the pollination by *gracilistyla* was practised, I got only nine *multinervis* (see Table VII), but then only a very few catkins were used for experiment, and it is hardly doubtful that had I experimented upon a larger number of catkins I should have been able to obtain a much larger *multinervis* progeny.

development only by its stimulating action (pseudogamy). As before stated, I have observed frequently in catkins covered with bags ovaries which have grown up somewhat more intensely than others and yet remain quite sterile; may not such ovaries be able to produce some seeds by the stimulus of foreign pollen? This is, however, a mere supposition, and the fact ought to be studied in future more in detail, because it is quite possible that exceptionally a much larger number of ovules than I was able to observe in my experiments might develop to seeds without any pollination.

The results of experiments of Pellew and Durham with *Primula verticillata*, *floribunda*, and the hybrid between them, *P. Kewensis*,¹ agree in several respects with what I have seen in our *Salix*. It appears that they have also met with autonomous as well as induced parthenogenesis, for they say,² 'We incline to suppose that the ovules are such that while they can occasionally develop without fertilization they more commonly develop in consequence of that stimulus.'

Concerning the above experiments of Pellew and Durham, Winkler makes the following remarks, which are reproduced below in his own words: 'Vor Allem aber ist . . . zu bedenken, dass die apomiktische Samenbildung auch ohne jeden Bestäubungsreiz erfolgen kann, so dass ein solcher als Auslösung der vermuteten Parthenogenesis jedenfalls nicht unentbehrlich sein, sondern höchstens fördernd wirken kann. Dann kann man aber auch nicht von einer durch die Bestäubung induzierten Parthenogenesis sprechen . . . sondern nur davon, dass durch den Pollenschlauchreiz die an sich schon vorhandene Neigung zur apomiktischen Samenbildung gefördert wird. . . .'³ All that Winkler says may be true, and also applicable to our case of *Salix*. To decide, however, experimentally the fact whether pollination has induced parthenogenesis, or has simply accelerated ('fördern') the tendency for apomictic development, would not be easy.

Had we to deal in our case with parthenogenesis, either autonomous or induced, the following remark might not be without some interest. It is clear that parthenogenesis has been originally derived from normal fertilization in the course of phylogenetic evolution. In respect to the manner of its origin there may be two possible ways. Firstly, certain animal or plant forms which have reproduced themselves at first by normal fertilization acquire at once the power of complete parthenogenesis by mutation, so that all eggs or ovules reach suddenly a condition such that they need no fertilization for their further development. Secondly, the transition from fertilization to parthenogenesis is gradual, i. e. several stages intervene between the two extremes—as we see, for instance, in some

¹ Journ. of Genetics, vol. v, 1916, pp. 159–82.

² l. c., p. 160.

³ Ursache und Verbreitung der Parthenogenesis im Pflanzen- und Tierreich, Jena, 1920, p. 175.

animals, as *Rhabditis aberrans* (Nematoda) studied by Krueger.¹ *Thalictrum purpurascens* as well as certain species of *Hieracium*, where some ovules are parthenogenetic and others normal, may be considered to be in their way of this transition.² Our *Salix multinervis* is also to be ranked among such transitional forms, and in view of the extreme rarity of parthenogenetic ovules, as well as of the fact that parthenogenesis is not always autonomous but in many cases induced (considering my supposition above expressed to hold good), we may regard our *Salix* as being in the very beginning of such transition.

The opinion was expressed by some authors that parthenogenesis has taken its origin in consequence of the abortion of pollen and the decline of sexuality, while that of others, especially Winkler, is quite opposed to it.³ Now if such were really the case we should also observe such phenomena in our *Salix*, but we can there recognize neither abortion of pollen nor decline of sexuality. For instance, Nohara has examined the germinating power of pollen of our *Salix* by the use of 2-3 per cent. cane-sugar solution, and found it to be as high as 98 per cent.⁴ I asked him to examine the germinating power of seeds of this *Salix* species, and he found that out of 721 seeds, taken from four catkins artificially pollinated by a male plant of the same species, 655 came to germination, i.e. 92.2 per cent. The results of these observations are obviously decidedly in favour of the opinion of Winkler.

We come finally to the problem of the sex of our apomictic progeny. As before stated, nearly fifty *multinervis* individuals resulting from the experiment in 1911 were female without any single exception; so also were two *multinervis* obtained by the pollination done in 1918 (cf. Nos. 26 and 29 in Table I). The sex of nine *multinervis* derived from the pollination made in 1919 (Table VII) is yet unknown. In view of the small number of the *multinervis* progeny resulting from the experiment of 1918, we are not able to make any inference about them, but the fact that all *multinervis* offspring derived from the experiment of 1911, numbering almost fifty, are female can hardly be without a certain significance. Before going farther, let us see first what we know about the sex of the progeny arising by parthenogenesis in animals and plants. In the former it is *arrhenotokous* (males produced exclusively), *thelytokous* (females produced exclusively), or *amphiterotokous* (both males and females produced) in different cases.⁵ Concerning a few dioecious plants where natural parthenogenesis has been discovered, the sex of the progeny arising by this process is known in

¹ Cf. Winkler, l. c., pp. 28 ff.

² Certain species of *Rubus* may also belong to such transitional form. (Cf. Lidforss, Zeits. f. induct. Abstamm.- u. Vererbungslehre, Bd. xii, 1914, pp. 1-13).

³ Parthenogenesis und Apogamie im Pflanzenreich, pp. 133 ff.

⁴ Bot. Mag., Tôkyô, vol. xxvii, 1913, p. 185.

⁵ Cf. Winkler: Ursache und Verbreitung der Parthenogenesis, p. 15.

some cases. Thus, for instance, in *Bryonia dioica* Bitter has obtained nine parthenogenetic plants, all of which were male.¹ In *Thalictrum purpurascens* the sex of the progeny due to this process is not specially mentioned.² In *Antennaria alpina* males are very rare, and parthenogenesis seems to be chiefly thelytokous.³ In *Chara crinita*, known long since as the classical example of natural parthenogenesis in plants, Ernst⁴ has recently discovered that there are two strains: in one, which reproduces itself by normal fertilization, males and females are produced in almost equal number, whilst in the other, which reproduces itself by parthenogenesis, plants are exclusively female, i. e. parthenogenesis is thelytokous. Since in our *Salix* species our repeated observations have proved that even in the case of normal fertilization females are much superior in number to males, the fact above enunciated, that all fifty offspring are female, might be due simply to the latter circumstance, inasmuch as had we had more than fifty plants it would have been possible that some male offspring might have been obtained. If, however, parthenogenesis in our *Salix* should prove really to be thelytokous, the fact is easily comprehensible, whether the female is homozygous or heterozygous in respect to the sex-determining factors; because, since in our case egg-cells are supposed to develop without undergoing any reducing division of chromosomes, they will remain always in the state of the so-called female-producing ones, *XX* in the first case and *WZ* in the second according to the well-known nomenclature of Morgan.

IV. SUMMARY.

1. By pollination of *Salix multinervis* by *S. gracilistyla* we get hybrids, as well as *multinervis* exactly similar to the mother-plant.

2. Two kinds of hybrids appear in F_1 . The one characterized by its densely hairy catkin is called *G-type*; the other characterized by its less hairy catkin is called *M-type*, and is produced in much less number than the other (for instance, 17 per cent. against 83 per cent. in round numbers). Though the two differ externally they are genetically equivalent.

3. From the study of various crosses we are led to the conclusion that the hairy catkin of *gracilistyla* is dominant as a rule to the less hairy one of *multinervis*. If we represent them by *D* and *R* respectively the F_1 progeny agree in being *DR*.

4. That in F_1 some *DR* individuals are *G-type* and others *M-type* is due to the imperfection of dominance: in a few cases dominance fails,

¹ Abhandl. d. Naturwiss. Vereins zu Bremen, Bd. xviii, Heft 1, 1904. Original not seen.

² Overton, J. C.

³ Juel: Kongl. Svenska Vetenskaps Akademiens Handlingar, Bd. xxxiii, 1900, p. 11.

⁴ Bastardierung als Ursache der Apogamie im Pflanzenreich, Jena, 1918. Cf. especially chapter iii, pp. 49 ff.

and the recessive character appears externally, giving rise to M-types. The appearance of two types in F_1 is not to be regarded as Mendelian segregation.

5. The segregation occurs first in F_2 as usual. The F_2 generation resulting from the cross between the F_1 progeny is variously composed: G-type ♀ × G-type ♂ produces chiefly G-types and a few M-types, M-type ♀ × M-type ♂ chiefly M-types and a few G-types, M-type ♀ × G-type ♂ both types in almost equal number.

6. If we add the results of the above three crosses together we find that we have G-types and M-types in the approximate proportion of 3:1, with a certain *positive* deviation on the side of the latter type: this deviation is due to the imperfection of dominance of the factor *D* in relation to the factor *R*.

7. The above conclusion has been fully confirmed by the results of back-crosses, either $DR \times R$ or $DR \times D$.

8. The degree of potency is inherited: i.e. G-type plants produce a much larger proportion of G-type progeny than do M-type plants, and vice versa.

9. Rarely *multinervis* × *gracilistyla* gives rise to *multinervis* progeny which breed true in later generation. Their production from a *multinervis* mother without any pollination at all has been also observed, though very rarely. Whether the embryo formation from nucellar cells or parthenogenesis is the real cause of such 'apomictic' development is not definitely established.

10. We have here to deal with parthenogenesis, which is very occasionally autonomous; in many other cases, however, it seems possible that it is induced by the stimulating action of foreign pollen (pseudogamy).

11. If parthenogenesis is derived phylogenetically from normal fertilization, and if this transition is gradual, our *Salix multinervis* may perhaps be regarded as being in the way of such transition, and in its very beginning.

12. Neither abortion of pollen nor decline of sexuality is to be detected in our *Salix*, which contradicts the view often expressed that parthenogenesis sets in in consequence of such circumstances.

13. All plants of *multinervis* produced by apomixis are female, without any exception. The explanation for it is given.

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The Fungus present in *Pellia epiphylla*, (L.) Corda.

BY

W. F. F. RIDLER.

With eight Figures in the Text.

MATERIAL of *Pellia epiphylla* gathered from Leigh Woods, Somerset, in 1920 was found to harbour a fungus. The following paper contains an account of an investigation of the life-history of the fungus—which always proved to be present in material collected from the locality mentioned above—as well as in material obtained from the Royal Horticultural Society's garden at Wisley, Surrey, where the plants had developed on humus at the base of an *Osmunda regalis*; from a wood at Pensford, near Bristol, where it grew on a soil mainly composed of clay; from the Jardin Botanique de l'État at Brussels—this material was removed from the 'Rouge Cloître' near Brussels in 1914 and since that date had been cultivated in a cold greenhouse; from Cromer, Norfolk; Worcester; Leeds; Belfast; and the Forêt des Soignes, Belgium. In the last case it was growing on sandy soil. With the exception noted above, the material was always from clay soils containing varying amounts of humus. No plant of *Pellia epiphylla* has been found in which fungal infection was entirely absent.

HISTORICAL NOTES.

Examples have been noted by various authors of species of Musci being infested by fungal mycelia. Thus Cooke (1889) recorded the presence of *Cladosporium epibryum* on certain moss plants without giving exact indications of the host on which they occurred. Britton (1911) obtained the list of mosses which are host species for this fungus from Massee, to whom they had been sent by Cooke. This list consisted of *Ulota phyllantha*, Brid., *Grimmia ovata*, W. and M., *Grimmia Doniana*, Sm., *Encalypta rhabdocarpa*, Schwgr., *Bartramia pomiformis*, Hedw., *Hypnum megaptilum*, Sull., *Fabronia andina*, Mitt., and *Bartramia Potosica*, Mont. In 1911 Györfy noted the presence of *Cladosporium herbarum*, (Pers.) Link., on capsules of *Buxbaumia veridis*, Brid. Dunham (1916) found the spores of *Pestalozzia* present in a capsule of *Funaria hygrometrica*, var. *patula*, Br. and Sch. Fitzpatrick (1918) published a detailed account of the

life-history and parasitism of *Eocronartium muscicola*, (Fries) Fitzpatrick, which he had found to occur chiefly on *Climacium americanum*, but which had also been collected on *Anomodon rostratus*, *Leskea obscura*, *L. polyantha*, *Thuidium delicatulum*, *T. minutulum*, *Amblystegium serpens*, *A. varium*, *A. riparium*, *Brachythecium oxycladon*, *Climacium dendroides*, *C. Kindbergii*, *Entodon seductrix*, *Hypnum chrysophyllum*, *Plagiothecium Muellerianum*, and *Pylaisia intricata*.

Servattaz (1913) recorded the occurrence of *Oospora* in connexion with the protonema of *Phascum cuspidatum*, Schreb.

Schimper (1858) and Warnstorff (1886) had observed the presence of numerous small spores in capsules of *Sphagnum*, among the spores of the moss. These they considered to be male spores. Nawaschin (1892) proved that these so-called 'microspores' were in reality spores of the fungus *Tilletia sphagni*. Warnstorff noted similar spores in capsules of *Pallavicinia Lyellii*, (Hook.) Gray. Cavers confirmed this observation (1903), and noted that the spores were abstricted from hyphae of the fungus and were therefore true conidia.

The occurrence of fungal hyphae in the tissues of Hepaticae was first described by Leitgeb, who observed that young sporogonia of *Ptilidium ciliare*, (L.) Hampe, were frequently infested by the mycelium of a fungus. Cavers (1903) recorded the occurrence of fungal mycelia in the sporogonia of *Lophocola bidentata*, (L.) Dum., *Radula complanata*, (L.) Dum., *Cephalozia bicuspadata*, (L.) Dum., and *Plagiochila asplenoides*, (L.) Dum. In the last two, fungal hyphae have also been observed in the gametophyte (see below). According to Cavers, fungal hyphae enter the fertilized archegonium from above, grow down the neck-canal, and, in some cases, enter the venter and pierce the egg. Infected sporogonia in these instances were usually imperfectly developed and remained abortive. If the capsules matured the cavities were filled with a mass of interlacing hyphae in which were embedded numerous small spherical bodies abstricted from the hyphae, and therefore regarded as conidia.

Kny (1879) observed that the rhizoids of *Marchantia* and *Lunularia* were frequently traversed by fungal hyphae. These possessed cross-walls, and occasionally branched, but did not reach the thallus tissue, except in plants growing on rich humus, when they entered the thallus and ramified through it.

Stahl (1900) referred to the occurrence of mycorrhiza in the Bryophyta. He considered that a real symbiosis may exist in the case of *Calypogeia Trichomanes*, (L.) Corda, and in other Jungermanniaceae, as plants harbouring hyphae appeared to be larger than plants possessing none. He indicated that the presence or absence of fungal hyphae might be determined by the soil, as plants growing on soils rich in humus contained more hyphae than plants growing on other soils. Stahl suggested that an actual

or a physiological shortage of water in the soil might lead to the presence of fungi in the thallus of *Musci*. Writing of the Hepaticae he connected the formation of starch in the thallus of the Marchantiaceae with a highly developed transpiratory organization and the complete absence, or at any rate meagre development, of a mycorrhiza. In the case of the Jungermanniaceae he connected the formation of sugar in the leaves with low transpiratory activity and the extensive occurrence of mycorrhiza. Since Stahl's paper was written, numerous examples of the presence of endotrophic fungi among the members of the Marchantiaceae have been recorded by Beauverie, Cavers, and Golenkin, and it is probable that the occurrence of fungi in the latter group is at least as frequent as in the Jungermanniaceae.

Beauverie (1902) described a fungus inhabiting the thallus of *Fegatella conica*, Corda. In this case the fungus produced both conidia and chlamydospores. Beauverie also suggested that a definite symbiosis existed, by means of which the life of the *Fegatella* plant became to a large extent saprophytic. Both in the plant and in cultures the fungus agreed closely with *Fusarium*.

Cavers (1903) described a fungus in the thallus of the New Zealand liverwort, *Monoclea Forsteri*. He found the fungus present in a sharply-defined zone of from two to four layers of cells in the thicker median portion of the thallus. The nuclei of the infected cells grew in size and became surrounded by tufts of short hyphal branches; the cells all contained chloroplastids, some of which also became surrounded by fungal hyphae. Large spherical vesicles, many of which had thickened walls, also occurred as in *Fegatella*. The fungus was not identified.

Golenkin (1902) described endotrophic mycorrhiza in *Marchantia palmata*, *Marchantia paleacea*, *Preissia commutata*, Nees, *Targionia hypophylla*, L., and *Plagiochasma elongatum*, as well as *Fegatella conica*, Corda. The hyphae in all cases were confined to the compact ventral tissue. The cells retained their nuclei and cytoplasm but contained no starch or chlorophyll. Golenkin suggested that the function of the fungus in these instances was to store water, thus rendering the plants more resistant to drought. Cavers pointed out that *Fegatella* and *Monoclea* are both hygrophyllous forms, and therefore this hypothesis seemed improbable.

Fungal hyphae have also been observed in the vegetative organs of many of the foliose Jungermanniaceae, including *Calypogeia trichomanes*, (L.) Corda, *Lepidozia reptans*, (L.) Dum., and *Lophozia bicrenata*, (Schmid.) Dum., by Němec; and in *Scapania nemorosa*, (L.) Dum., *Diplophyllum albicans*, (L.) Dum., *Bazzania trilobata*, (L.) Gray, *Porella platyphyllum*, Lindb., *Cephalozia bicuspidata*, (L.) Dum., and *Plagiochila asplenoides*, (L.) Dum., by Cavers. The two last also possess fungal hyphae in their sporophytes as noted above.

Němec (1899) identified the fungus in *Calypogea trichomanes* as an Ascomycete, *Mollisia Jungermanniae*. This fungus bore bluish-green apothecia, and covered the plant with a web-like mycelium. Where the mycelium penetrated the cells of the leaves or stem the cells lost their protoplasm and became discoloured.

Humphreys (1906) recorded the occurrence of tuberous swellings on the stem of *Fossombronia longiseta* which contained a fungus, but he neither described the fungus nor indicated its relationship to the plant.

Czapek (1889) stated that the tissues of *Fegatella*, *Marchantia*, and *Lunularia* contained an antiseptic substance, 'sphagnol', which existed in combination with the cell-walls and exerted an inhibitory influence on the growth of bacteria and moulds. Cavers has suggested the view that the sphagnol may serve to regulate the growth of the fungus, and to prevent symbiosis from passing into parasitism.

Coulter, Barnes, and Cowles (1911) referred to the occurrence of endotrophic fungi in mosses and liverworts, and various theories were mentioned by them to explain the significance of this symbiosis. They noted that in the Bryophyta fungal symbiosis seems to cause diminished rather than increased luxuriance and that probably the fungus alone is benefited.

W. G. P. Ellis (1897) described a disease caused by a fungus on *Pellia epiphylla* in the Botanic Gardens, Cambridge. The fungus produced a cobweb-like mycelium over the thallus and fructifications consisting of branched aerial conidiophores bearing clusters of round conidia. Septate hyphae resembling those on the outside of the thallus were also found in the cells of the plant, chiefly in the uppermost layers of cells, except in what are termed the 'rejuvenation shoots'. Cultures were made from spores removed from the spore clusters on the host, on nutrient gelatine; these grew to form mycelia which produced similar fructifications. Spores from these latter were used to inoculate the sterile apices (or rejuvenation shoots) of plants of *Pellia epiphylla* growing in the infected area, also other plants which were brought from a distance, and showed no trace of the disease. The spores put out germ tubes which penetrated the upper epidermis of the host and entered the interior. Ellis found that the fungus did not enter by the rhizoids, but only through the upper surface of the thallus. The walls of infected cells became brown, the protoplasm shrunken, the chloroplastids lost their colour and became massed together. Ellis identified the fungus as the conidial form of an Ascomycete, similar to, if not identical with, the *Trichoderma*-phase of *Hypocrea*. There are several important differences between the chief characteristics of the disease described above, and those of the subject of the present investigation. In the latter the fungus enters by the rhizoids, is present only in the lower portion of the thallus, and is never in the upper superficial layer or the one

immediately beneath. No mycelium bearing conidia like those described by Ellis have been observed on the *Pellia* plants which have been used for the present paper; moreover, infected material remains quite green and appears perfectly healthy. It is therefore clear that Ellis was dealing with a fungus quite different from the one which forms the subject of this paper. He did not notice our fungus apparently.

H. E. Greenwood (1911), in a paper, 'Some Stages in the Development of *Pellia epiphylla*', described in great detail the structure and life-history of the plant, but no mention of the presence of any fungus was made.

DISTRIBUTION OF THE FUNGUS.

A. In the Gametophyte.

Transverse sections of the thallus indicate that the fungus occurs only in the lower portion of the thickened central region.

The fungus is most easily seen in longitudinal sections of the thallus through the 'midrib', which, in this species, is normally from ten to twelve cells in thickness. The number of fungal hyphae present does not vary according to the habitat of the *Pellia*, but rather according to the degree to which the infection of the thallus has proceeded. As the plant is perennial this depends mainly on the season of the year.

In new growths branching from the thallus, only the rhizoids and lower epidermis contain the fungus; in older branches the two or three layers of cells adjoining the ventral surface as well as the rhizoids are infected, and in mature branches eight or nine layers of cells are inhabited by the fungus; the upper two or three layers of cells, including the upper epidermis, remain free from hyphae. In one or two cases only the upper epidermis remained absolutely free of fungal hyphae—all the other cells were infected.

The hyphae apparently enter the thallus through the rhizoids. Two or more hyphae may pass up one rhizoid; in a few cases these show ladder-like fusions (cf. Němec, *Calypogeia trichomanes*), but more often remain quite separate. Cross-walls occur at rather long and irregular intervals. The fungus branches as soon as it reaches the thallus, but sometimes not until it enters the layer of cells immediately internal to the lower epidermis. In the cells of the thallus the fungus branches freely, ramifies through the thallus, and extends to within about 2 mm. of the growing-point (Fig. 1).

In some cells the ends of the hyphae become considerably swollen. These swellings occasionally occur also in intercalary positions, but they are usually terminal. They possess very granular contents, are thin-walled, and are probably merely swellings of the vegetative mycelium, which may be regarded as storage organs (Fig. 1). Large spherical and in some cases oval-shaped bodies also occur on the hyphae (Fig. 1). These

are always terminal and possess very thick walls and granular contents, and measure $21-28\ \mu$ in diameter. They apparently correspond to the vesicles described by Cavers in *Monoclea Forsteri*, and by Beauverie in *Fegatella conica*. Beauverie regarded them as chlamydospores. This, in all probability, is their nature, as they occur in very large quantities in the old parts of the thallus—that is, in the portions which die off. As decomposition of these old portions of thallus proceeds, the spores may possibly enter the soil and germinate. In the plant, however, they have only been

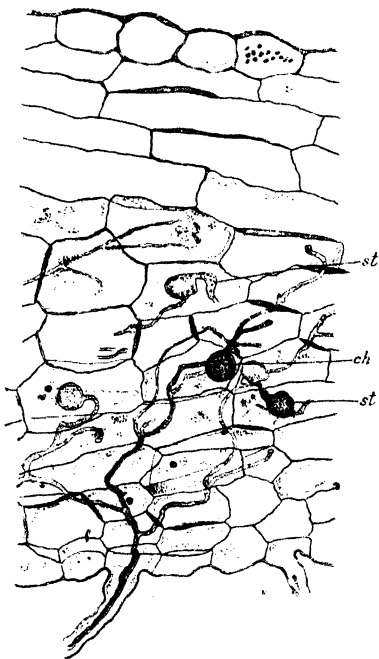


FIG. 1. Longitudinal section of thallus through the thickened median portion, showing hypha from a rhizoid branching, ramifying through the thallus, and bearing storage organs and chlamydospore. $\times 125$. *st.*, storage organ; *ch.*, chlamydospore.

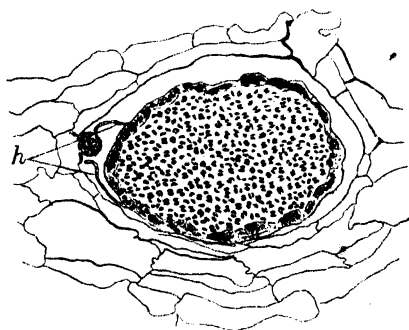


FIG. 2. Longitudinal section through the thallus, showing an almost mature antheridium with fungal hyphae around it. $\times 125$. *h.*, hyphae.

observed in connexion with the mycelium, and in various attempts which have been made to isolate the fungus from the thallus they did not germinate.

The hyphae in the rhizoids vary to some extent in appearance. They may be very narrow, with thin walls, and constricted at short intervals, or they may be very narrow and thin-walled, but not constricted. These forms occur in the younger rhizoids. In older rhizoids the hyphae are much wider, with granular contents and thick walls which are often brown in colour (Fig. 1). This is the normal condition for hyphae in the tissue of

the thallus itself, where they measure from $5-7\ \mu$ in diameter. In the younger branches of the mycelium the finer, thin-walled type occurs again. These hyphae measure $1-4\ \mu$ in diameter. The hyphae are very rarely constricted in the thallus except in the region of the antheridium (see below). In only one case have constricted hyphae been observed in the thallus.

In older plants heavily infected by the fungus, cells occur, dark brown in colour, in which the fungus produces curious structures somewhat resembling oogonia and antheridia. No further stages have been observed, however, and it is possible that they are merely indications of the hypertrophy of the vegetative mycelium.

In several cases the fungus has been observed in proximity to antheridia (Fig. 2). The fungus enters through the aperture of the

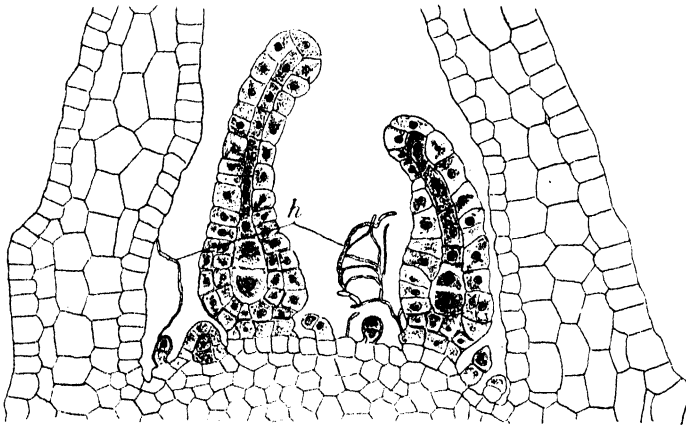


FIG. 3. Longitudinal section through the thallus with archegonia, showing fungal hyphae in close proximity to them. $\times 125$. *h.*, hyphae.

cavity. No direct connexion has been observed so far between hyphae in the thallus and in the antheridium. The fungal hyphae in the latter are often constricted similarly to those in the rhizoids. When the antheridium is mature, and the antherozoids have escaped, the hyphae fill the empty antheridial cavity.

Fungal hyphae, similar to those around the antheridia, have also been observed near unfertilized archegonia (Fig. 3).

B. In the Sporophyte.

The fact that the fungus was present in the sporophyte was not observed for some considerable time. Normal healthy sporophytes were repeatedly examined, and no sign of the fungus was discovered in foot, seta, or capsule.

However, while the setae of the healthy sporophytes were elongating and the capsules dehiscing it was noticed that on other plants, much discoloured owing to extreme infection by the fungus, capsules were present

which, instead of being the usual dark-green colour, were brownish-black, and moreover the setae of these did not elongate. On examining sections of this material it was found that the whole of the sporophyte as well as the thallus contained very great quantities of fungal hyphae. A series of sections showed that very fine hyphae penetrate from the thallus to the



FIG. 4. Longitudinal section through the thallus in the region of the foot. Fine hyphae are passing from the thallus into the foot. $\times 125$. *f*, foot; *th*, thallus; *h*, hyphae.

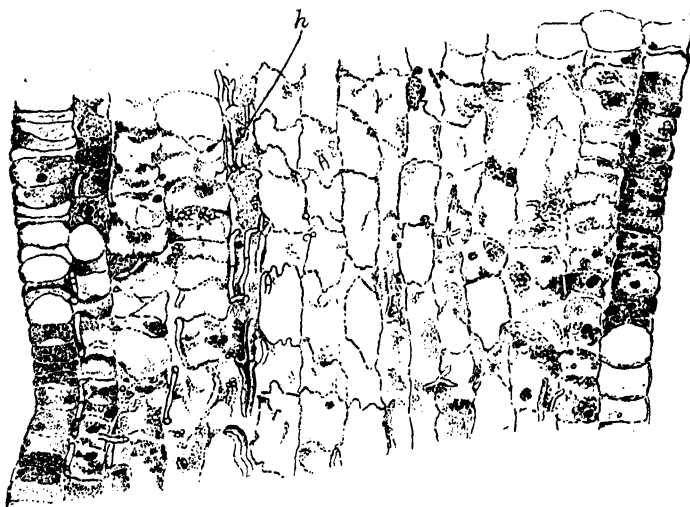


FIG. 5. Longitudinal section through the seta of an infected sporogonium. Some of the cell-walls are partially destroyed. $\times 167$. *h*, hyphae.

base of the foot (Fig. 4), and enter, ramifying through the tissue of this organ. The hyphae then pass up into the seta, where they become very much wider, possibly owing to the rich store of accumulated food material

at their disposal (Fig. 5); they then enter the capsule (Fig. 6), ramifying between the young gametophytes and in some cases actually entering them (Fig. 7). There is also a considerable quantity of hyphae in the capsule-wall, in the calyptra, and in the involucre.

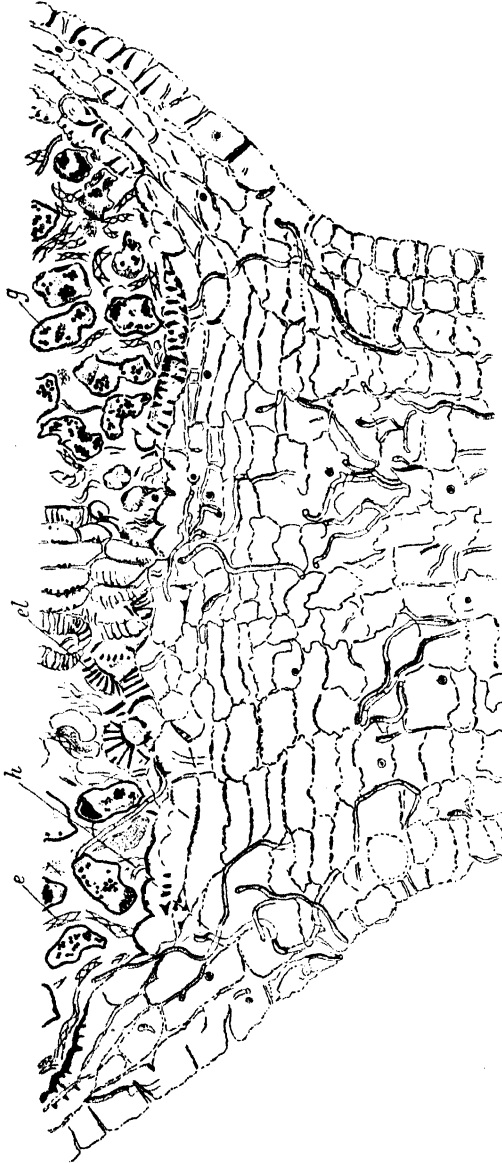


FIG. 6. Longitudinal section through part of the seta and capsule of an infected sporogonium, showing hyphae passing from the seta into the capsule. $\times 125$. *h.*, hyphae; *el.*, elaterophore; *g.*, young gametophyte; *e.*, elater.

The hyphae in the sporophyte possess cross-walls at regular intervals, and the walls are not quite so thick as those of the usual type in the gametophyte; probably this also is due to the presence of the rich food

store. On the capsule-wall, on the calyptra, as well as on the involucre, small pycnidia are produced containing numerous conidia (Fig. 7).

The pycnidia are from 80–120 μ in diameter, smooth, globose, and open at the apex by a pore. They are completely or, more frequently, partially embedded in the tissue of the plant. The conidia are hyaline, one-celled, cylindrical in shape, 5–6 μ long and 2 μ wide.

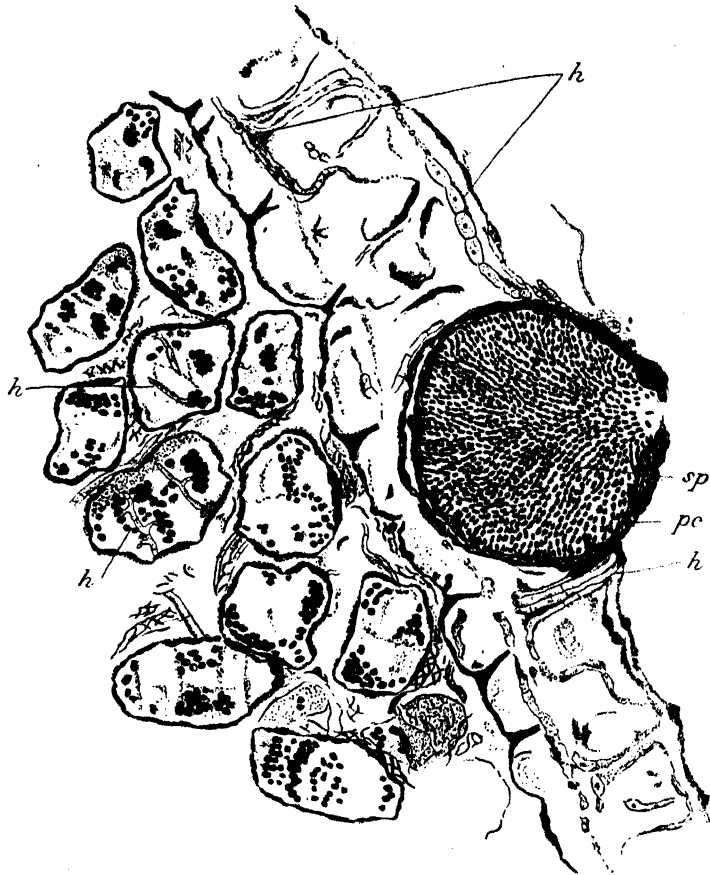


FIG. 7. Longitudinal section through part of the capsule of an infected sporogonium, showing hyphae inside the young gametophytes, and a pycnidium produced on the wall. $\times 333$. *h.*, hyphae; *pc.*, pycnidium; *sp.*, conidia.

Certain sections showed that the fungus had penetrated to the foot and seta, but had not yet entered the capsule, the spores of which were still green and uninjured. In other sections through the sporophyte it was seen that though there was a considerable number of fungal hyphae in the seta and capsule, and around the foot, there was none in the gametophyte thallus in the neighbourhood of the sporogonium. The capsules in such cases had not fully developed; the capsule-walls were not fully differentiated,

the elaters had not developed spiral thickenings, and the spores had only reached the tetrad stage.

One sporogonium was found in a still more aborted form. In this case only a few divisions had taken place in the fertilized egg, which contained fungal hyphae, though there were none in the thallus near it.

Apparently, therefore, there are three methods by which the fungus may enter the sporogonium :

(a) Hyphae from the thallus penetrate to the base of the foot, enter it, and ramify through the tissue of the sporophyte. In this case the spores of the liverwort may form young gametophytes before the fungus obtains a hold on the capsule, but they will not grow farther.

(b) Hyphae may enter the fertilized archegonium and ramify through the developing sporogonium, which in this case remains abortive to a varying degree, its development ceasing almost at the beginning, the sporogenous tissue reaching the spore mother-cell stage or the tetrad stage—but the spores never germinate within the capsule.

(c) The hyphae may enter in both the above ways. Here, too, the sporogonium usually remains abortive.

ISOLATION OF THE FUNGUS.

Repeated attempts were made to isolate the fungus from the thallus of the liverwort. Portions of the thallus were broken off, teased out with a sterilized platinum needle, sterilized in a 0.1 per cent. solution of mercuric chloride, and placed on plates containing various nutrient media. The fungus, however, did not grow. The experiments were repeated without sterilization in mercuric chloride solution, it being thought that the latter process might have killed the fungus, but no better success was obtained.

Various species of *Penicillium*, *Eurotium*, *Mucor*, &c., as well as *Bacteria* were obtained on some of the plates in the latter case, whilst others remained sterile, but the endophyte was inactive. Meat agar, *Pellia* extract agar, *Pellia* extract gelatine, potato glycerine agar, malt agar, synthetic media with the addition of dextrose, or dextrose and peptone, and beerwort agar were used in these trials. Some cultures were incubated at a temperature of 23° C. ; others were kept in darkness at room temperature.

Hanging-drop cultures were also tried. Rhizoids containing fungal hyphae were removed from the thallus, and placed in drops of various media (beerwort, *Pellia* extract, and distilled water). These were examined under the microscope at intervals, but, although in some cases fungal hyphae were seen emerging from rhizoids, they did not develop farther.

Upon the discovery of fungal hyphae and spores in the sporophyte, attempts were again made to isolate the fungus. Pieces of infected capsules were placed by means of a sterilized needle on agar plates.

Within twenty-four hours the spores were germinating vigorously, and cultures of the fungus were obtained. These were reinoculated on various media.

IDENTIFICATION OF THE FUNGUS.

The fungus isolated in this way is a species of *Phoma*. It has not been found possible to reinoculate with it, as no *Pellia* without the fungus has been found. Attempts were made to propagate the *Pellia* alone from the sterile apices of plants, but without success. Young gametophytes of

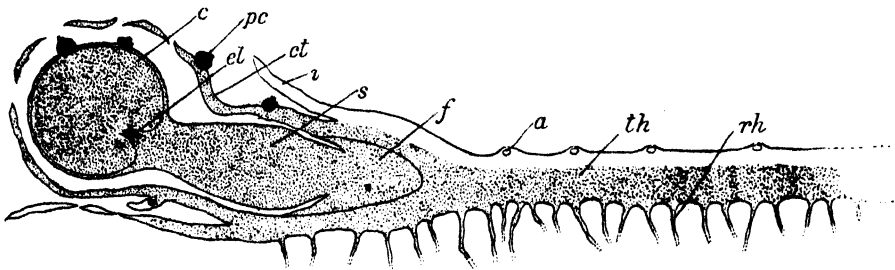


FIG. 8. Schematized drawing of a longitudinal section through the thallus and sporogonium. Shaded portion indicates the infected region. $\times 15$. *c.*, capsule; *pc.*, pycnidium; *el.*, elaterophore; *ct.*, calyptra; *s.*, seta; *f.*, foot; *a.*, antheridium; *th.*, thallus; *rh.*, rhizoid.

the liverwort have been placed under similar conditions, but did not develop to any extent.

PHYSIOLOGICAL RELATIONSHIPS.

The Gametophyte. The effect of the fungus on the tissue of the gametophyte is well marked. When first infected the cells of the thallus possess numerous chloroplastids and well-marked protoplasmic contents. As infection proceeds, the cells become brown and discoloured, and the chloroplastids disappear from all but the two or three layers of cells nearest the upper epidermis, which are free from the fungus. In a well-infected thallus the lower part becomes absolutely brown; the difference between this infected zone and the upper layers is, in some cases, very marked, the latter being still quite green and the contents of the cells uninjured.

The fungus in the brown zone is obviously in a flourishing condition, as are also the swollen vesicles. The cell-walls of the liverwort are apparently quite uninjured by the fungus, except at the point of penetration.

The presence of sphagnol in the cell-walls was tested for, but none was present. In this case there is therefore no evidence of an inhibitory influence exerted on the growth of the fungus, which may be the reason why it is able to obtain such a hold on the plant.

The relationship between the fungus and the gametophyte may be one of symbiosis. The apparently constant occurrence of the fungus in the

thallus, and the difficulty of isolating it, point to such a relationship. The fungus evidently finds the liverwort a very favourable substratum, as indicated by the considerable number of fungal hyphae found in some material, branching profusely, and ramifying through the tissue in all directions, and obtaining food material from the cells into which they have penetrated.

The plant is to some extent unharmed—at least it can go on growing and reproducing in the normal manner. Whether the fungus is of any actual use to the liverwort is at present uncertain. Upon entering the thallus the fungus seems to sever its connexion with the soil, so that it is unlikely that the association is a mycorrhiza.

Whatever may be the relationship, it seems probable that the fungus is the dominant partner, obtaining its food material from the liverwort, damaging it to a certain extent by killing the cells which it enters; but the liverwort, in most cases, still retains the power of growth and reproduction.

In a few instances, however, the association becomes of the nature of a disease. Here the cells are quite brown in colour; the thallus in consequence, instead of appearing green, is dark brown, and incapable of further development.

The Sporophyte. In the sporophyte the effect of the fungus is much more drastic than in the gametophyte. The fungus has a twofold effect upon the tissues. The cells are turned brown in colour, the chloroplastids are destroyed, and the cells are ultimately killed. Secondly, the cell-walls partially disappear; this is especially noticeable in the capsule-wall, where only the thickened walls of the cells are left whole, the thin walls being very indistinct or completely absent. The young gametophytes also are killed or prevented from reaching their full development. Their cell-walls are partially or completely absorbed, and the chloroplastids are discoloured, and all attempts to make them germinate, even when fully developed, have failed, whereas similar spores from healthy capsules germinated readily on a synthetic agar medium. Moreover, the whole tissue of the sporophyte becomes infected, as there is no definite zone which remains free of hyphae, as in the upper layers of cells in the gametophyte.

The cell-walls of the sporophyte did not respond to the test for sphagnol.

The relationship between the fungus and the sporophyte is obviously not one of symbiosis: in this generation of *Pellia* the fungus is a disease, killing the tissues and rendering them incapable of maturing.

SUMMARY.

1. The cells of the thallus of *Pellia epiphylla* contain a fungus which occurs in a definite zone along the thickened median portion towards the ventral surface of the thallus and in the rhizoids.

The fungus also occurs in proximity to the antheridia and archegonia.

2. In some cases the fungus is present in the cells of the sporophyte, where it may infect the whole of the tissues, sometimes rendering them abortive.

3. The fungus has been isolated from the sporophyte, and identified as a species of *Phoma*.

4. The effect of the fungus on the gametophyte of *Pellia* is very marked. The protoplasmic contents of the infected cells are killed, the chloroplastids disappear, and the cells ultimately become brown in colour.

The relationship existing between the fungus and the liverwort may be a symbiotic one; but the *Phoma* is probably the dominant partner and of little use to the *Pellia*—in extreme cases killing the latter, though usually it is able to grow and reproduce in the normal manner.

5. The effect of the fungus on the sporophyte is twofold. The contents of the cells are killed, and the cell-walls are also wholly or partially absorbed.

The relationship existing in this case is not symbiosis. The fungus causes a disease, killing the tissues of the sporogonium and in some cases rendering them abortive.

The courtesy of Professors Massart, Priestley, and Small, in providing material for this work, has been of great value in proving that the presence of the fungus in *Pellia epiphylla* is not restricted to a limited locality, but that it is normally present in plants obtained from such different sources.

I wish to express my sincere thanks to Mr. C. Hunter, who suggested the subject of this paper, and whose constant valuable help and advice alone have made it possible to carry out this investigation.

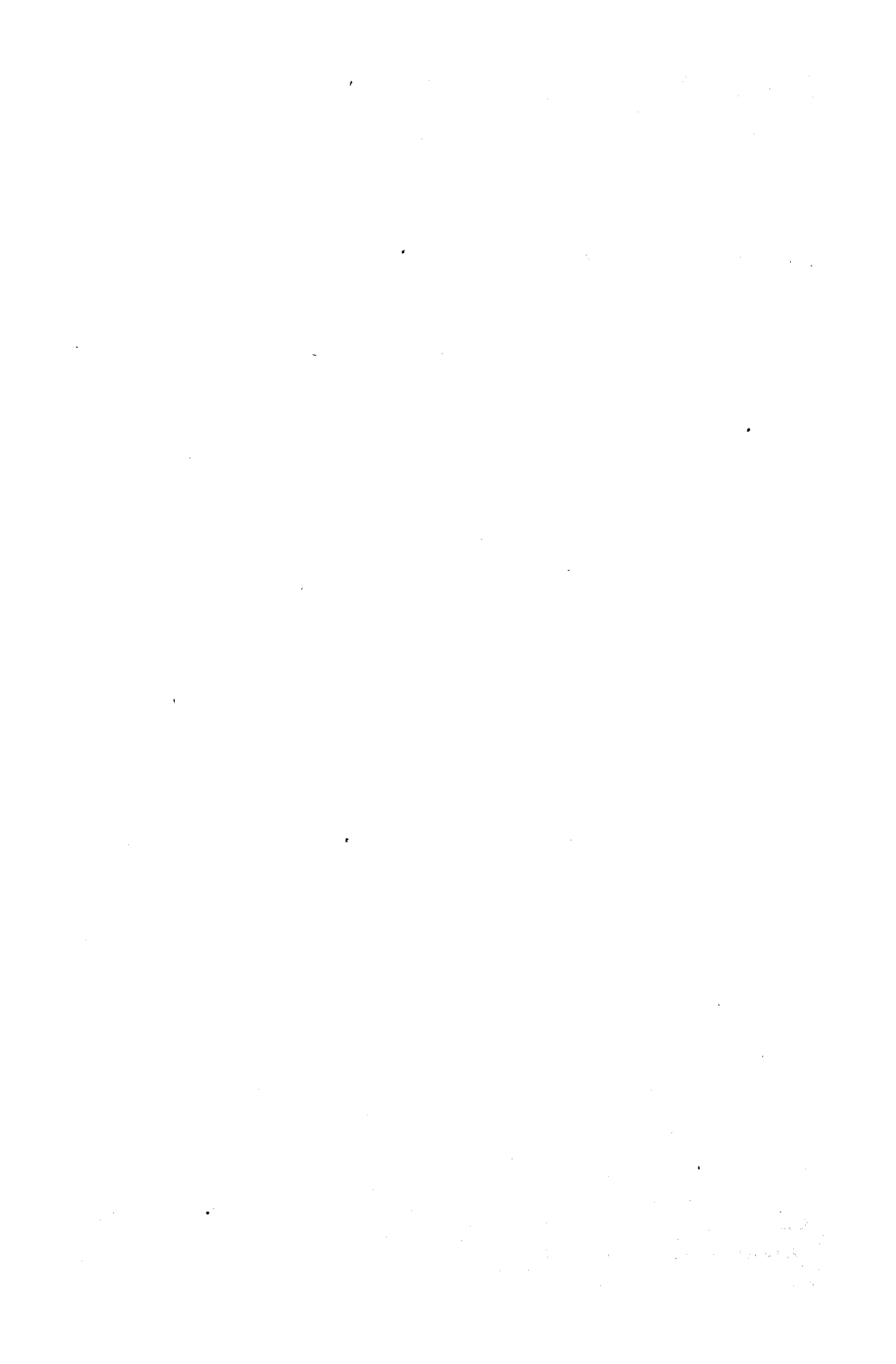
I also wish to thank Professor O. V. Darbishire for his advice and interest in this work.

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November 1921.

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The South-east African Flora: Its Origin, Migrations, and Evolutionary Tendencies.

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INTRODUCTION.

FEW regions are more favourably situated than South-east Africa for the study of plant distribution and its bearing on questions of evolutionary history. It is being recognized that one of the main problems in connexion with the phylogeny of the Angiosperms is the relationship between the tropical, subtropical, and temperate floras as well as the origin of various ecological types. Now in South-east Africa within a comparatively small area there occur (1) a purely tropical flora, which is a southerly extension of the tropical flora of Central Africa on the coast-belt; (2) a derived subtropical flora, adapted to drier and cooler climates, which becomes increasingly prominent towards the south on the coast-belt and occupies most of the region of rising altitude (1,500 feet and upwards) away from the sea; and (3) a mountain or temperate flora, which occupies the higher altitudes (8,000 ft. and over it) on the Drakensberg and other mountain ranges, descends to sea-level in the south-western region of the Cape, and connects through the mountains of Central Africa with the temperate flora of the Northern Hemisphere. The relationship between the first two types is very close. The purely tropical species are in a very large number of cases closely allied to the frost-resisting or more xerophytic subtropical species, and the tropical-subtropical vegetation may be grouped together as one element of the South African flora. The various modifications of it in reaction to changed ecological conditions, e. g. succulence, spinescence, and other forms of xerophytism, herbaceous growth-forms with capsular fruits and many other types, afford abundant opportunity for one line of research. A comparison of the coast-belt species with their nearest allies in the mid-lands of Natal from the morphological and physiological standpoints is in progress, and the results already obtained are extremely interesting.

The temperate or mountain flora is a very distinct element. Of course it mixes to a certain extent with the tropical-subtropical element, but, in the

main, even the families represented are different. The relationship of this flora to the tropical is much the same as that of the great temperate flora of the Northern Hemisphere, and will be discussed more fully later.

Instead of beginning with general comparisons and attempting to work downwards to details, it would appear more promising and more in accord with the usual methods of science to begin at the other end and study the migrations of the flora in a smaller area, afterwards extending the observations to larger and larger areas. As far as South Africa is concerned, the present study of plant geography in the larger sense has gradually developed out of detailed ecological investigations. Movements of species were analysed in connexion with plant succession, various lines of migration were traced, at first somewhat hypothetically, but the hypotheses were tested by an appeal to the facts of distribution, and now the final attempt is being made to connect up the various lines of migration so as to obtain a complete picture. The accepted principles of evolution in floral morphology have been used to check the results, but, on the other hand, the history of general plant migrations may be used to throw some light on doubtful points in evolutionary history. The most logical arrangement would be to begin with the tropical flora, give the reasons why the sub-tropical should be considered derivate, and then trace as many of the changes as possible. I prefer, however, to follow the order in which this investigation was actually carried out, since it gradually developed out of other work.

RIVER VALLEY MIGRATION.

The rivers of South-east Africa flow in a general easterly direction from the Drakensberg to the sea. The deeply cut river valleys are dry with extremes of temperature, and the vegetation is xerophytic grassland, thorn veld, or succulent scrub, the most extreme types being on the south sides of the valleys which face the sun. Along the upper flanks, especially on the north side, mesophytic forest develops. There are also various types of rocky scrub and tree veld on the flanks of the valleys. In brief, the various trees and shrubs (including at least a thousand species) of South-east Africa, composing the woodland plant communities, are, on the whole, associated in their distribution with the river valleys and their slopes.

The first detailed study of plant succession was made in the thorn veld (1). It was afterwards seen that the other types of parkland tree veld were similar. The pioneers are trees. They migrate out from the river banks, and colonize the grassland areas, and are followed by other more mesophytic trees and shrubs.

This, however, is the last step in the process of migration. The bases of colonization are the various river-bank woodland communities which extend for long distances along the river. To carry the process a step

farther back, therefore, it appears clear that there is extensive migration along the rivers either up or down the valleys. The conditions, of course, are uniform, but it is worth noting that nearly all the African trees and shrubs have fruits and seeds that are distributed by animals, especially birds, and these also feed and migrate along the rivers. Succession in dense mesophytic forest differs in detail from that in tree veld, but it also follows the rivers and their tributary streams (for details see 3). The same applies to other subordinate types of scrub.

We thus reach a first generalization that for trees and shrubs (mostly distributed by animals) composing various types of woodland (thorn veld, mesophytic tree veld, succulent scrub, rocky hillside scrub, dense mesophytic scrub and forest) migration takes place most rapidly in a direction parallel to the river valleys. This, we shall see, applies chiefly to the midlands of Natal, where the river valleys are deeply cut, and many species seem to have difficulty in crossing the intervening ridge between one river valley and the next. This hypothesis has now to be tested by reference to the distribution of such trees and shrubs as have not spread all over the eastern side of South Africa. We find a large number of such relatively rare species confined to the coast-belt, and their lines of maximum dispersal and therefore presumably of their migration run parallel to the coast, but such species are for the most part purely tropical or slightly modified subtropical.

In the Midlands, on the other hand, almost without exception, the rarer species have a maximum distribution parallel to the river valleys and ridges and at right angles to the coast-line. As an example, the Tugela Valley and its tributaries may be chosen. The following species are confined to it, and have not, so far as we know, as yet been able to cross into the next main river valley of the Umgeni farther south: *Croton rivularis*, *C. menyharti*, *C. zambesicus*, *Vitex mooiensis*, *V. rehmanni*, *Heeria paniculosa*, *Euphorbia tugelensis*, *Ipomaea albivenia*, *I. oblongata*, *Convolvulus ulosepalus*, *Ceropegia barklyi*, var. *tugelensis*, *Fockea tugelensis*, *Raphionacme flanaganii*, *Viscum pulchellum*, *V. subserratum*, *Pouzolzia* sp., *Boerhaavia bracteata*, *B. repens*, *Clematis glaucescens*, *Capparis calvescens*, *Acacia robusta*, *Rhus excisa*, *Pavonia urens*, *Royena simii*, *R. scabrida*, *Mimusops oleifolia*, *Olea enervis*, *Strychnos dyssochila*, *Lippia scaberrima*, *Lycium pendulinum*, *Pavetta gerrardi*, *Melothria maderaspata*, and others. Many of these are true endemics, others occur farther north, e. g. in the Transvaal but not elsewhere in Natal. In high forest, along the flanks of the ridges, the component species are usually more widespread, but there are examples of new endemic and more mesophytic species which spread most rapidly in a direction at right angles to the coast, e. g. *Podocarpus henkelii*. The river valleys and intervening ridges, while serving as lines of migration, also serve as barriers to migration across them.

The vegetation of the Tugela and its tributary, the Lower Mooi River, though, as the above list shows, it is rather distinct from that of the rest of Natal, has certain fairly close connexions with that of the dry kopjes in the northern Transvaal, e.g. *Heeria*, *Vitex*, *Croton*, *Pappea*, *Ficus*. For a time this appeared somewhat of a distributional puzzle. The two areas are separated by a great stretch of high veld grassland, many hundreds of miles wide, where these species do not occur. The explanation is suggested when we observe that the Northern Transvaal bears the same relationship to the great valley of the Limpopo as the Lower Mooi River area does to the Tugela. The species in question spread along both of these main river valleys. It is clear that this river migration must be connected up with the general coast-belt invasion of tropical and subtropical species from the North.

COAST-BELT MIGRATION.

As we have already pointed out, the maximum distribution of the coast-belt species is in a line parallel to the coast. The whole flora has close tropical affinities, but the actual tropical species diminish in numbers towards the south.

At the outset it is necessary to distinguish clearly between seashore migration and that of the rest of the coast-belt. Strand plants and seashore sand-dune species, as well as mangroves and other plants of the mud lagoons, whose seeds are capable of withstanding submergence in sea water, are rapidly and widely distributed, as Guppy has fully demonstrated (5). In South Africa the southern limit of the mangroves and other tropical forms in Tembuland is probably determined by the increasing influence of the cold shoreward current from the south, which flows northwards as a counter-current to the warm Mozambique current from the north. The latter is, of course, so much larger that it warms the whole eastern side of South Africa and raises the average temperatures much above those of corresponding latitudes on the western side. The cold counter-current varies in strength at different times and in a way not thoroughly understood. Occasionally its influence is very marked, as when it kills the fish of the tropical waters with which it mixes, and these float ashore in shoals. It is just such extreme and exceptional occurrences that are of the greatest importance in their effect on the vegetation.

While the actual coast-line is a uniform easy pathway leading to rapid migration for its own characteristic flora, farther inland on the coast-belt migration is slower, and rather pronounced changes in topography and soil conditions have tended to impose a check on the invasion of many species. Reference to any physical map of Africa will show that the 1,000 foot coast-belt, which is several hundreds of miles broad in Portuguese East Africa, becomes narrowed like the neck of a bottle at Port Durnford in Zululand.

Northward from that point it is a broad, flat, sandy belt usually only 100 feet or so above sea-level, but in Natal it becomes confined to a strip a mile or two broad along the coast-line. A considerable number of tropical species have not penetrated farther south than Zululand, and this is due probably rather to the altered topography than to any general lowering of temperature, though the cold current already referred to makes its influence felt as far as Port Durnford, where the outward bend in the coast-line turns it round. The coast-belt flora is purely tropical to the north of Zululand, but in Natal and in the eastern coast-belt of the Cape it has produced large numbers of derived endemic species. Mr. R. D. Aitken is carrying out a statistical analysis of these on the lines adopted by Willis. Such coast-belt endemic species as remain confined to the frost-free localities differ but slightly from the tropical 'wides'. Other subtropical species are more modified and penetrate farther into the colder and drier areas.

Along the frost-free localities on the flanks of the river valleys the tropical flora of the coast-belt has tended to migrate often far inland. *Dichrostachys nutans*, for instance, a characteristic and often dominant tropical tree veld species, has penetrated as far as the Lower Mooi River area. Recently, while exploring the Umgeni Valley, I was much interested to find, at altitudes of 3,000 feet, such typical coast-belt species as the um Doni (*Eugenia cordata*, which is the dominant species in hygrophilous coast-belt bush), *E. gerrardi*, *Chaetacme aristata*, *Dracaena hookeriana*, and *Iboza riparia*.

Comparatively few, however, of the actual tropical and coast-belt species thus migrate inland. The flora of the Midlands and the Cape eastern coast-belt, in response to lower temperatures and drier conditions, has been modified and consists of species often closely allied to but usually not identical with those occurring in the tropics, or if they do occur farther north it is at higher and higher altitudes the nearer to the Equator. We can now complete a portion of the main picture.

Purely tropical vegetation consisting largely of mesophytic trees and shrubs with usually simple leaves has invaded South-east Africa along the coast-belt and, with diminishing numbers, has penetrated even into the Cape Colony south of Natal. Some of the species have migrated far inland along the flanks of the river valleys. From this purely tropical vegetation an allied modified subtropical vegetation has been derived, which has penetrated south as far as the limit of the area of summer rainfall (beyond Port Elizabeth) and has migrated inland at right angles to the coast-line along the river valleys and intervening system of ridges. The subtropical flora is adapted, on the one hand, to lower temperatures and, on the other hand, to drier conditions. This has led to a considerable diversity of growth form. A succulent habit is a very common result. Thorn development in response to dry conditions is very marked. Evidence is accumulating to show that

compound or divided leaves are far more common in derived subtropical species than in the purely tropical. The herbaceous habit is often derivative.

MIGRATION AND EVOLUTION: COMPARISON OF TROPICAL AND SUBTROPICAL FORMS.

The accepted principles of phylogeny on the whole support the view adopted, viz. that the subtropical flora has been derived from the tropical, but, as we shall see later when the temperate element is also compared, floral evolution has not always run parallel to that of the vegetative morphology. An examination of practically all the families has been made, but considerations of space prevent our dealing with more than a few comparisons here. The following families are chosen at random:

Flacourtiaceae. The tribe Erythrospermeae with the perianth leaves spirally arranged is the older and widespread in the tropics, having one genus, *Rawsonia*, which reaches Natal. The other nine South African genera are subtropical and more advanced in flower structure, having perianth leaves whorled and usually petals present as well as sepals.

Violaceae. The tropical-subtropical Rinoreae (shrubs and trees with nearly regular flowers) are to be contrasted with the subtropical and temperate Violeae, herbs with irregular flowers.

Loranthaceae. *Loranthus* has an hermaphrodite and less reduced, and therefore being parasitic probably an older, type of flower than *Viscum*. *Loranthus* is distinctly more tropical than *Viscum*.

Thymelaeaceae. *Octolepis* is the most primitive type, with a flat receptacle, and is purely tropical (seven species in West Africa). *Peddica* is an East African tropical-subtropical genus with a two-celled ovary and fruit a drupe, not so primitive as *Octolepis*, but more so than all the other South African genera of shrubs, undershrubs, and herbs which have the ovary one-celled. There are certain pairs of families which might be compared and contrasted to illustrate the same thing, e. g. Apocynaceae, more tropical and relatively more primitive, and Asclepiadaceae, distinctly more subtropical or even temperate and relatively more advanced in floral morphology. Araliaceae and Umbelliferae, Myrsinaceae and Primulaceae are similar pairs.

Euphorbiaceae. This family is clearly tropical in origin, and the most recent and highly developed derivative types like *Euphorbia* have penetrated farthest into the colder and drier regions, while the tropical types, though they have broken up into great numbers of distinct genera, have retained more of the primitive floral characters. The genus *Euphorbia* with about 1,000 species has a remarkably uniform floral structure, and very extreme vegetative variation from tiny annual herbs up to large trees over sixty feet high. Variations in floral structure are confined to the involucre

glands, which, however, grade into one another completely, and sometimes vary in the same species or on the same individual. The systematic works say little or nothing regarding the probable evolutionary history of the genus, but if we follow it step by step along the lines of its invasion into South Africa we can give the following outline of its evolution. The more mesophytic tropical species of shrubs with erect, leafy, woody, spineless stems probably come nearest to the ancestral form. Some of them have invaded Eastern South Africa, e.g. *E. epicyparissias*. The purely herbaceous type has been derived, but has not diverged very far. Of the herbaceous forms the small annuals are most recent, and of these *E. inaequilatera* is one of the commonest and has spread all over Africa. Many are weeds of cultivation, e.g. *E. peplus* and *E. helioscopia*. A section of the perennial herbaceous types has developed tuberous root-stocks. The shrubby types have in another direction developed spines which, according to N. E. Brown (4), are of three types in the South African species: (1) where the apex of a branch becomes spiny, as in *E. lignosa* and *E. spinea*, two dwarf shrublets from Namaqualand (one or two transitional forms have tapering branches not acutely spine-tipped); (2) where the peduncle becomes transformed into a spine; and (3) the so-called 'stipular spines', which are in pairs but are probably not stipular in origin.

Spine development, as we have noted, is one of the general reactions to drier conditions. The main evolutionary tendency in the genus, however, has been towards succulence. It is seen in the large variety of low-growing forms that have probably developed from the perennial herbaceous forms and in the large succulent tree Euphorbias which come nearer to the primitive shrubby forms. The succulent, 'leafless', and often spiny Euphorbias have developed from the tropical types partly in the dry areas of the western side and partly in the dry river valleys of the eastern side where the species are usually distinct. The Karroo species are also distinct.

In support of the view that the numerous succulent South African species are recent and derivative we have not only the general origin of the subtropical South African flora, but the further fact that when cultivated under moist conditions succulent species show a tendency to revert to a shrubby type and develop slender leafy branches with no trace of succulence. This has been noted particularly by N. E. Brown in connexion with *E. gorgonis* as cultivated at Kew (see 4, and Gardeners' Chronicle, 1914, lvi. 230, fig. 91, p. 312).

Though succulence, thorn development, and other xerophytic characters are common features of derivative subtropical forms, it must not be assumed that evolution in the South African flora has always been in this direction. There are numerous extra-tropical mesophytic forest situations where recent endemic forms have been produced. Some genera show development in both directions.

Gymnosporia (Celastraceae). The oldest type of this genus, according to inflorescence and fruit characters, has no spines, and is represented in South Africa by such a type as *G. acuminata*, a small tree which occurs usually outside but sometimes inside forest. *G. peduncularis* is a closely allied large, mesophytic, forest tree. *G. cordata*, also near the ancestral type, is a coast forest species. A more recent type, with the inflorescence in clustered cymose panicles, is represented by *G. buxifolia*, a somewhat variable and widespread species all over South Africa. It is very spiny in the drier situations and illustrates the development, on the one hand, towards xerophytism. On the other hand, the rare endemic, *G. amapondensis*, is a recent type with the fruit one-celled instead of three-celled. It is known only from the Egossa forest in East Pondoland and illustrates the development towards mesophytism.

Any other large genus which is tropical and subtropical can be dealt with in exactly the same way as in the case of *Euphorbia* and *Gymnosporia*.

SUBTROPICAL GRASSLANDS.

The flora of the great subtropical grassland areas consists of species which, unlike the trees and shrubs, are mostly wind-distributed, and the origin and migrations of the type as a whole are not so easily traced. The origin of the grasses themselves is also somewhat obscure. In the Bamboos the grass flower approaches nearest to the ordinary monocotyledonous type, and possibly they are the most primitive. The twenty-three genera of Bambuseae are mainly tropical with a tendency to extend largely into the mountains of the tropics, but they are not grassland types. The tropical and subtropical grasslands are dominated mainly by genera belonging to the tribes Andropogoneae and Paniceae, where the spikelets are much reduced and highly specialized, phylogenetically an advanced type.

Aristida (Stipeae) is a large important genus adapted to drier subtropical and desert conditions.

Temperate grasses, on the other hand, belong mainly to the tribes Aveneae, Festuceae, and Hordeae, with spikelets less specialized and containing usually numerous florets. This would seem to suggest that, with the exception of the Bamboos, the temperate grasses have retained more of the primitive floral characters than the tropical, but there is little agreement on the course of evolution in the grass flower, and the subject must for the present remain obscure.

In South-east Africa the most distinctly tropical type of grassland is that of the coast-belt, where there is an admixture of species such as *Pollinia villosa*, *Perotis latifolia*, and species of *Panicum*, but the dominant species belong to the Andropogoneae, as in the high veld and low veld areas all over the eastern side. Species of *Aristida*, *Eragrostis*, and *Sporobolus* show adaptation to drier conditions and open formations. At

higher altitudes there is an admixture of temperate species of *Poa*, *Festuca*, &c., but all the eastern grassveld remains on the whole subtropical.

Mixed with the grasses throughout there are enormous numbers of herbaceous or shrubby species. The 'autumnal aspect societies', which tend to replace the grasses, are again tropical in their affinities. On the other hand, the great mass of 'vernal aspect societies', which are bulbous or geophytic as a rule in their growth forms, belong to such families as the Compositae, Papilionaceae, Geraniaceae, Asclepiadaceae, Liliaceae, Amaryllidaceae, Iridaceae, and, while constituting a special type of their own, the species being largely endemic, they have closer affinities with the temperate flora than with the tropical. They or allied species may occur on the mountains and elevated grassy plateaux of the tropics, but they are not characteristic of the tropical forest regions.

It is interesting to note that these vernal aspect societies are characteristic of early stages of the plant succession, being gradually suppressed as succession advances. I have pointed out elsewhere that in a subtropical region, as succession advances, the vegetation tends to become more and more tropical, e. g. on the Natal coast-belt (3).

THE TEMPERATE OR MOUNTAIN FLORA: MIGRATION ALONG MOUNTAIN RANGES.

Reference once more to a physical map of Africa will show that the whole eastern side is a region of elevation. Some of the river valleys, e. g. the Zambesi and Limpopo, have cut rather far back through the mountain escarpment of the inland plateau, but otherwise highly elevated land is continuous from Abyssinia to the Drakensberg and westward across the southern end of South Africa to the Cape Peninsula. The south-western region of the Cape with its winter rainfall and dry summers has a 'Mediterranean flora' of warm temperate rather than tropical affinities. This temperate flora at increasingly high altitudes is continued eastward through the Drakensberg in Natal and the Transvaal and northward through Central Africa to Abyssinia. It is not only distinct ecologically but also floristically from that of the tropics and subtropical eastern side.

The Compositae, Ericaceae, Proteaceae, Rosaceae (*Cliffortia*), Geraniaceae (*Pelargonium*), bulbous Monocotyledons, and many distinctive sections or genera in other families are most prominent in this mountain flora. The absence or rarity of such families as Acanthaceae, Capparidaceae, Anonaceae, Menispermaceae, the majority of the Euphorbiaceae, large sections of the Leguminosae, the Sapindaceae, Melianthaceae, Sapotaceae, Cucurbitaceae, and many others so prominent on the coast-belt is equally striking. The majority of the species of the temperate or mountain flora are either herbaceous or low-growing shrubs often gnarled and twisted. Mountain regions are regions of unstable topography and variable climatic conditions. Some

situations are very moist, others very dry; some are shady, others fully exposed to the intense light of high altitudes; some are free from frosts owing to the rapid cold air drainage, others near at hand are not, and all these varied types of habitat are usually thoroughly mixed up in any small area.

Judging from the number of endemic species characteristic of mountain ranges, such variable and unstable conditions are favourable for the production of new species. These may be very rare, but in other cases mountain species extend for immense distances along the ranges without descending to lower altitudes. Mountain ranges are, therefore, looked upon as great highways of migration for their own characteristic flora, and further, like the river valleys, they act as barriers to migration across them.

There is considerable difference of opinion regarding the origin of the temperate African flora. It is richest in numbers in the south-west of the Cape Colony, a region climatically most suited to it, and there it occurs down to sea-level. Eastward, as soon as the region of summer rainfall is entered, it becomes entirely a mountain flora. It was first investigated at the Cape and is best known there. It is natural to speak of the occurrences of *Ericas*, *Proteas*, &c., on the Drakensberg as 'outliers' of the south-west flora, a term which, to a certain extent, assumes an origin for it in the south-west. It has connexions with the flora of Australia and South America, and it is therefore looked upon as the remnants of the flora of a former temperate Antarctic continent. It is not, on the whole, phylogenetically an old flora in spite of assumptions to the contrary, and it is extremely doubtful whether any Antarctic continent has existed since the rise of the Angiosperms.

Other authorities believe that the mountain and South-western African flora has come from the north. The original immigrants travelled south along the mountain ranges crossing the Equator, and when they reached the more temperate south-western areas developed enormously and produced the great numbers of new species which now occur there.

It is not, of course, necessary to assume single centres of origin for any of the widespread component elements of this flora (such as the *Ericaceae*). So long as we deal with the larger groups such as the families and large genera there are many reasons for believing 'multiple origins' or polygenesis as the most likely, and the recent developments of genetics show that the polygenesis of species is also extremely probable. Without, therefore, arguing further concerning the exact geographical origin of the temperate African flora, we may turn to the question of real interest, viz. its relationship to the tropical-subtropical flora, with which geographically it is so closely associated.

Sinnott and Bailey (6) have brought forward much evidence from palaeobotany, anatomy, and phylogeny to show that the tropical woody

type of plant is an older form than the temperate herbaceous type. In addition to the evidence from other branches of botany, their argument from the geographical standpoint is summed up as follows: 'There is great preponderance of herbs in temperate regions and of woody plants in the tropics. The latter climate probably approaches more nearly to that under which Angiosperms first appeared. Herbs have a short life cycle and are therefore able to survive periods of cold underground or in the form of seeds. Their great development in temperate regions has probably been in response to the progressive refrigeration of the climate during the course of the Tertiary.'

Though there are a great many herbs (especially bulbous Monocotyledons) in the temperate African flora, there is also a high proportion of small woody shrubs, and the number of endemics is exceptionally large. Now Sinnott and Bailey argue that the endemic plants in a flora are to be regarded usually as its most ancient element, a conclusion exactly the opposite of that reached by Willis (8). Sinnott and Bailey would have it that the herbaceous element of the temperate African flora is recent and derivative, while the woody element is ancient, a conclusion which receives no support from its present-day distribution, nor from the phylogeny of the families to which most of the woody types belong, Compositae, Ericaceae, Proteaceae, &c. The same areas are occupied by both elements, herbs and woody shrubs. Before discussing these views further we may investigate what is to be learned by comparing the temperate and tropical floras.

COMPARISON OF TEMPERATE AND TROPICAL FLORAS.

Certain families have already been compared as regards their tropical and subtropical representatives. The conclusions there reached, viz. that the tropical types were older than the subtropical, apply also in many cases when the former are compared with temperate types. This is the case in the Violaceae, Loranthaceae, Thymelaeaceae, Apocynaceae, and Asclepiadaceae taken together, and similarly the Myrsinaceae and Primulaceae and Araliaceae and Umbelliferae, each pair taken together. In other families the same thing is seen, the more primitive section in floral characters being tropical, the more recent temperate, e.g. Boraginaceae, Santalaceae, Ulmaceae, Liliaceae, Rubiaceae, Leguminosae, and others. It is noteworthy that the more primitive tropical types usually have fleshy indehiscent fruits, while the more recent temperate types have usually capsular fruits.

There are, however, a considerable number of families in which the temperate representatives have apparently retained more of the primitive ancestral characters in their floral morphology. In the Verbenaceae the subfamily Stilboideae has usually nearly regular corollas and endospermic seeds, and is more temperate than the Verbenoideae, which has irregular corollas and exendospermic seeds, or than the other subsections, the Viticoideae

and Avicennioideae. The same thing is seen in the Iridaceae, the temperate forms or at least the south-western Cape forms having more primitive floral characters than the eastern and subtropical. The Orchidaceae, with the exception of the Diandrae section, have also, on the whole, retained the less highly developed types of floral structure in the temperate representatives. The Gramineae have already been dealt with from the same standpoint.

Phylogeny, therefore, does not support so definitely the deriving of the temperate flora from the tropical as it did in the case of the more closely allied subtropical. The temperate and tropical floras have probably both diverged in different directions from the ancestral forms. At the same time it is probably true, as Sinnott and Bailey maintain, that the tropical climate approaches more nearly that in which Angiosperms first appeared. Consequently the tropical flora has remained more primitive on the whole in its growth forms, which are usually of a woody mesophytic type with simple bifacial leaves.

The herbaceous form is on the whole derivative, the shorter life cycle being better adapted to colder seasons and drier situations. The bulbous and tuberous type with underground storage has been multiplied in an enormous series of forms in response to grassland conditions. Capsular fruits and wide dispersal of seeds have tended to replace fleshy fruits and animal dispersal in many families.

Various kinds of xerophytism, epiphytism, parasitism, and the aquatic habit are generally derivative. Leaf division is probably, on the whole, also derivative and relatively recent in the Angiosperms.

Evolution in floral morphology, however, has not always been parallel to evolution in vegetative form. In some cases the tropical representatives of a group are the most primitive in flower characters, in other cases not. It should not be forgotten that genera, and, where possible, species also, are named by the systematist on floral characters. Under favourable moist, warm conditions the tropical flora, while retaining a fairly uniform type of growth form, has broken up into an immense number of new and probably often fairly recent floral forms, i.e. genera and species. Each of these is rather rigid in its requirements, and shows little plasticity, though germinally each type may be relatively unstable and ready to break up into further new forms. At any rate, tropical vegetation is, as is well known, exceedingly mixed and the numbers of species extraordinarily great. The temperate flora, on the other hand, has produced a much greater variety of growth form, but has a smaller total number of species. Each type is individually more plastic. A succulent, for instance, will grow quite well under moist conditions if competition with other plants is removed, but a moist tropical species can endure neither low temperatures nor dry conditions.

While it is not safe to assume that the tropical flora has produced the temperate or vice versa, while it is better to consider that both have diverged

from the ancestral forms, yet there are many examples in the larger genera of a tropical or subtropical genus, e. g. *Rhus*, which has produced temperate representatives, or of a temperate or mountain genus, e. g. *Pelargonium*, which has invaded the tropics. The whole family of the Compositae are, according to Small (7), probably montane and temperate in origin, yet they have invaded the tropics in considerable numbers. By following the probable migrations of a genus among the mountain forms, as was done in the case of *Euphorbia* and *Gymnosporia* among the tropical-subtropical forms, interesting light is thrown on its evolutionary history.

Pelargonium, unlike *Euphorbia*, has somewhat variable floral characters, the only really constant generic character being the uppermost segment of the calyx forming a 'nectariferous tube', adnate to the pedicel. The petals vary in number (5, 4, or 2) and size, and from being subequal to very unequal. In the androecium there are ten filaments, but only from seven to two are fertile. Harvey has broken up the genus into fifteen sections, a division which other systematists have adopted, e. g. Knuth in 'Das Pflanzenreich', p. 53, 1912. The section *Hoarea*, consisting of stemless tuberous-rooted species, are placed at the beginning as section 1, but though the section is a large one, and all typically south-western, it is doubtful whether it should be considered the most primitive. The fact that it has a relatively narrow range of distribution would tell against its primitiveness, according to Willis's Age and Area law (8). The section *Eumorpha* has a wider distribution from Abyssinia to South Africa. Allied to it is the section *Peristera*, in which the petals are minute, scarcely longer than the calyx; the calyx tube is sometimes nearly obsolete, e. g. in *P. fumarioides*, which is almost an *Erodium*; the habit is herbaceous like that of a *Geranium*. This section is the most widely distributed of all, extending all over Africa and having one species in India and two in Australia. The central species in South Africa is *P. grossularioides*, which is found all over the Cape and is a mountain species in the Drakensberg. Other species of the section occur in Namaqualand and the north-west. Considering carefully these facts of distribution it would appear probable that the ancestral form was a slightly woody or suffruticose type which probably arose somewhere in the extra-tropical mountain ranges. The woody habit was emphasized in many south-western forms, especially in the section *Pelargium* to which the well-known *P. cucullatum* belongs. Succulence either of stems or leaves is another common feature as an adaptation to drier conditions. The stemless tuberous-rooted sections *Hoarea* and *Seymouria* are all south-western.

The point of chief interest regarding *Pelargonium* is the way in which, though extra-tropical in its origin, it has produced many species which find a place in tropical or subtropical areas. I have listed seventeen species of *Pelargonium* in my 'Flora of Natal and Zululand' (3). Two are marked

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'doubtful', five are purely mountain, but the others are found either in the Midlands or even on the coast-belt. *P. aconitiphllum* is the commonest. It is, like the others, adapted to grassland conditions, and forms one of the commonest of the 'vernal aspect societies' in subtropical grassveld. This illustrates again the temperate affinities of those vernal aspect societies.

In the above account the South-east African flora as a whole has been dealt with in a general way, and the details mentioned have been used as examples. More detailed investigations on the above lines, in which our modern knowledge of morphology, physiology, and physiological anatomy is being utilized, are in progress at this centre, and the results appear likely to be very interesting.

SUMMARY.

1. South-east Africa has a flora composed of two distinct elements: (a) a tropical-subtropical element, and (b) a temperate or mountain element. The study of various lines of migration throws light on the origin of these elements and also on many questions of evolutionary history.

2. The various trees and shrubs composing the woodland plant communities are distributed mainly by birds and other animals, and they tend to migrate most rapidly along the main river valleys at right angles to the coast. This river valley migration, however, is the final step in a general migration from the north along the coast-belt.

3. The tropical flora has invaded South-east Africa and remains distinctly tropical on the coast-belt. The numbers of tropical species diminish southwards, being gradually replaced by allied subtropical species. A few of the tropical species have migrated for considerable distances inland along the valleys, but in general with rising altitude, lower temperatures, and drier conditions the flora becomes subtropical.

4. A comparison of the floral morphology in allied tropical and subtropical forms shows that the former is older and the latter derivative. Succulence, spinescence, and other forms of xerophytism in response to drier conditions are characteristic of the subtropical flora, as is illustrated by the evolution of the genus *Euphorbia*. Sometimes derived and recent species may be, on the one hand, more xerophytic and, on the other hand, more mesophytic, as in the genus *Gymnosporia*.

5. The species composing the South-east African grasslands are mostly wind-distributed and lines of migration are not so easily determined. The grasses are tropical or subtropical and also the autumnal aspect societies. Vernal aspect societies, however, while largely endemic, and of a type by themselves, have on the whole more affinity with the temperate element of the flora.

6. Great mountain ranges run parallel to the eastern coast of the African continent across the tropics and connect with the south-western

Cape region of winter rainfall. These form a great line of migration for the temperate or mountain element of the flora, which bears a relationship to the tropical flora much the same as that of the northern temperate regions. Mountains are regions of unstable topography and great climatic variations, conditions which appear to favour the production of new species.

7. A comparison of the floral morphology in allied tropical and temperate forms shows that in many families the tropical element is the older, but in others the reverse is the case, the temperate representatives having retained more of the primitive ancestral floral characters. It is suggested that the tropical flora has remained more primitive on the whole in its growth forms, which are of a rather uniform, woody, mesophytic type with simple bifacial leaves. Herbaceous forms, bulbous and tuberous forms, capsular fruits, plants with divided leaves, xerophytes, &c., are in general derivate and more characteristic of the temperate flora. Floral evolution and vegetative evolution have not always run parallel.

8. While it is considered advisable not to attempt to derive the temperate flora as a whole from the tropical or vice versa, yet examples are given of tropical genera producing species which have invaded temperate regions, e.g. *Rhus*, and of temperate genera which have invaded the tropics, e.g. *Pelargonium*.

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Growth Studies.

I. A Quantitative Study of the Growth of Roots.

BY

J. H. PRIESTLEY and A. F. C. H. EVERSLED.

With five Tables and six Figures in the Text.

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I. INTRODUCTION.

DURING the last few years an extensive re-exploration of the problem of growth has taken place in which quantitative methods have been widely employed. We shall not attempt any summary of this work, as the present state of our knowledge has recently been presented very fully, especially in a series of papers by Briggs, Kidd, and West (3 and 11).

Modern quantitative studies of plant growth are mainly based upon data as to changes in volume or length, whilst animal physiologists measure the growth of animals in terms of increase or decrease in length. This difference in attack is simply determined by the nature of the experimental material. Any investigator who has made efforts to record the growth of a baby or any other active young animal will know that length measurements demand a more than expert manipulation. On the other hand, if plant weights are to be significant, they must be dry weight determinations, and then the possibility of the progressive record of the growth of the same individual plant is removed.

Quantitative work upon the growth of animals appears to have been more fruitful in interpretation, and the reason seems to be that the records are of increase in mass and not in area or length. With the plant, change

in form or size may be the expression of such subsidiary influences as intake and retention of water rather than of cumulative increase in living substance resulting from constructive metabolism. The aim of the work now presented was to obtain data for plant growth based upon mass measurements, with a view to seeing if the subsequent analysis of such data would be of value in throwing any light upon the inner mechanism of growth.

2. EXPERIMENTAL MATERIAL AND METHODS.

Choice of Material.

Need for quantitative data seems to have become apparent to many workers recently, and from Cambridge (Briggs, Kidd, and West (3)), the Imperial College (Gregory (4)), and Rothamsted (Brenchley (2)) valuable results have been published since our experiments began in the summer of 1920. Most of these figures refer either to the growth of the *whole plant* or to the growth of *leaves*.

In choosing our experimental material it was necessary to bear in mind the fact that the leaf area represents the proportion of the plant engaged in the manufacture of food from raw materials. Figures giving an increase in leaf area would be expected to demonstrate the existence of an exponential law, because, as V. H. Blackman (1) has recently pointed out, increase in mass is directly connected with increase in photosynthetic area. Such an exponential relation between mass increase and time would depend upon a different train of circumstances from that connecting animal growth with time.

In order, therefore, to find out if a similar relationship between growth and time existed in the case of plants, attention was restricted to *roots*, where increase in manufacturing surface does not directly follow upon increase in mass, and where conditions more approximate to those obtaining in experiments with animals. Seedlings were not used for the preliminary work, on account of the difficulty of securing a uniform start, as germination involves a number of factors, including the resistance offered by the seed-coat, its gradual decomposition by bacteria, the original rate of entry of water, &c. These obstacles were all removed by the decision to obtain data as to the *production of roots upon cuttings*.

Experimental Method.

In this work, first with *Tradescantia Zebrina* and later with tomatoes (*Solanum Lycopersicum*), we endeavoured to obtain *uniformity of conditions* between the cuttings of each set of experiments. The cuttings, taken from plants grown in the same greenhouse, were of approximately *equal weight*, and were all started at the same time. At definite time intervals, the root production was measured in terms of wet weight, and then of dry weight.

Generally ten determinations were made on each occasion, and the probable errors estimated (Wood (12)).

We are aware that these experiments are on a relatively small scale, but circumstances make it impossible for us to extend their scope appreciably. However, with regard to the experimental results and their value as a basis for new conclusions, it must be noted that the significant feature of the curves appears in every set of figures and in every case is associated with the same phenomenon. This characteristic feature remains when full allowance is made for the calculated probable error of the data, at the critical points of the curves.

We hope that later further investigations, with increased experimental facilities, will permit of our conclusion receiving critical revision.

3. EXPERIMENTAL RESULTS.

A summary of the results of our experiments will be found in the following tables and curves (Figs. 1-4). Brief details as to the conditions under which each set of growth data were obtained are given. The most significant figures are given by the *Tradescantia* experiments, and reference to the tables shows that this material has yielded much more uniform results and that the experimental error is relatively small.

Data obtained from the experiments with tomatoes, however, are valuable in that they show, as the most striking features in a somewhat erratic curve (Fig. 4), the same characteristic points as in the case of *Tradescantia*.

Experiments with Tradescantia.

Series I. The cuttings were started on June 5, 1920, and were taken from plants in the greenhouse at Weetwood Hall and grown in empty flower-pots which stood inverted in dishes of water. The cuttings selected were as uniform as possible, their weights varying between 2.5 and 2.8 grammes, and the number of leaves on each varying from 5 to 8. Ten cuttings were placed in each pot, and there were 20 pots in all. At intervals, one shoot was taken from each pot, and the roots were removed with a sharp knife, dried on blotting-paper, then weighed, dried at 100° C. and weighed again. No roots were visible on the cuttings until the night of Thursday, June 10, and the first estimations of root-weight were made on June 14. Subsequently the remaining cuttings were taken at various dates, but the last twenty not until July 5.

The dry weights and wet weights of the twenty cuttings were recorded individually, and then the mean weight and probable error calculated.

In the following table, only the mean weights and probable errors are given from consideration of space; for the same reason, temperature and humidity records of the greenhouses are not given, though they are available,

together with full notes as to weather conditions during the time of the experiment.

TABLE I. *Tradescantia*, Series I (see Fig. 1).

Number of days' growth.	Mean wet weight of roots.	Mean dry weight of roots.
	gram.	gram.
9	0.07 ± 0.01	0.004 ± 0.001
11	0.15 ± 0.01	0.009 ± 0.002
12	0.17 ± 0.03	0.011 ± 0.002
13	0.19 ± 0.03	0.015 ± 0.003
14	0.21 ± 0.03	0.018 ± 0.003
17	0.21 ± 0.04	0.019 ± 0.003
19	0.31 ± 0.07	0.023 ± 0.004
23	0.42 ± 0.10	0.041 ± 0.009
29	0.56 ± 0.10	0.045 ± 0.011

Series II. In this series, the cuttings were grown *separately* in bottles of about 600 c.c. capacity. The bottles were filled with the three-salt

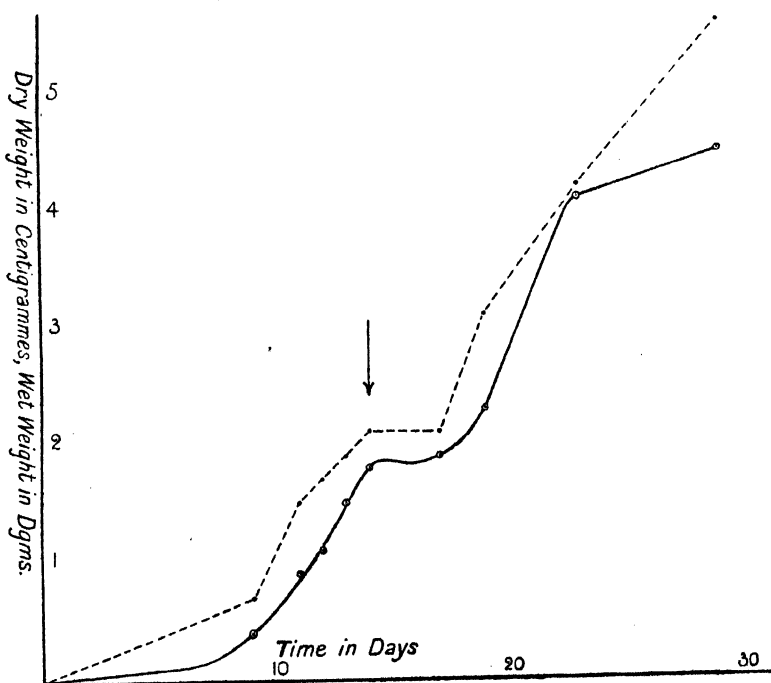
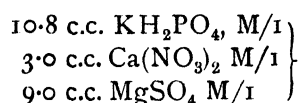


FIG. 1. The increase in weight of roots on cuttings of *Tradescantia*. The dotted line gives the data for wet weight on a reduced scale. The arrow shows when secondary roots appeared.

solution found by Shive (10) to be most suitable in his investigation of water-culture methods. Gram-molecular solutions were made up of potassium dihydrogen phosphate, magnesium sulphate, and calcium nitrate. These

solutions were kept in large stock aspirator bottles, which were permanently connected to burettes with three-way taps.

To each 600 c.c. bottle were added :



and the bottles were then filled up to the neck with tap-water.

To each bottle were added seven drops of a fine suspension of ferric phosphate (0.2 grm. in 100 c.c. distilled water). The bottles in this experiment were placed in a specially constructed lead-lined wooden trough, in which they stood immersed in water above the shoulders; the necks protruded through holes in black paper so that the roots developed in the dark. The water in the trough was kept continually flowing, and throughout the experiment the temperature remained very constant. The trough was placed on a laboratory table in a room with a glass roof, through which the cuttings obtained very satisfactory illumination. In warm weather the floor and table were sprayed several times daily to prevent the atmosphere from becoming too dry, and the plants throughout the experiment remained very healthy.

112 bottles were accommodated in the trough, and the cuttings were started in them on July 13. They were as uniform as possible, and their weights varied between 3.5 and 4.5 grammes.

The roots were collected and weighed separately, ten cuttings being taken on each date.

The first roots were removed on July 22 and the last set were weighed on September 7.

The culture solutions were renewed at intervals. The mean weights and probable errors are given in the following table, and the results are plotted in Fig. 2.

TABLE II. *Tradescantia*, Series II (see Fig. 2).

Number of days' growth.	Mean weight of wet roots. gram.	Mean weight of dry roots. gram.
9	0.08 ± 0.01	0.005 ± 0.001
11	0.14 ± 0.03	0.008 ± 0.001
13	0.19 ± 0.05	0.010 ± 0.002
14	0.22 ± 0.04	0.012 ± 0.001
15	0.32 ± 0.05	0.017 ± 0.001
17	0.31 ± 0.04	0.016 ± 0.002
21	0.43 ± 0.04	0.024 ± 0.002
24	0.64 ± 0.06	0.034 ± 0.003
34	0.75 ± 0.09	0.038 ± 0.004
45	0.89 ± 0.11	0.043 ± 0.006
56	0.84 ± 0.13	0.042 ± 0.009

In this series the secondary roots were first observed on July 26 and

were apparent on all roots by July 27. On August 6, after 24 days' growth, the first signs of tertiary roots were visible on most of the plants.

Series III. These experiments were carried out under the same conditions as Series II, only that the cuttings belonged to two groups, 45 cuttings having a mean weight of 5 gm., and 45 of 2.5 gm. No roots were removed until after 50 days. The series was started on August 19 and the first roots collected on October 8. Only five cuttings were taken each time, from each set, so the probable error is larger, but the experiment was primarily intended to show the effect of the original *mass* of the cutting on the later part of the growth curve.

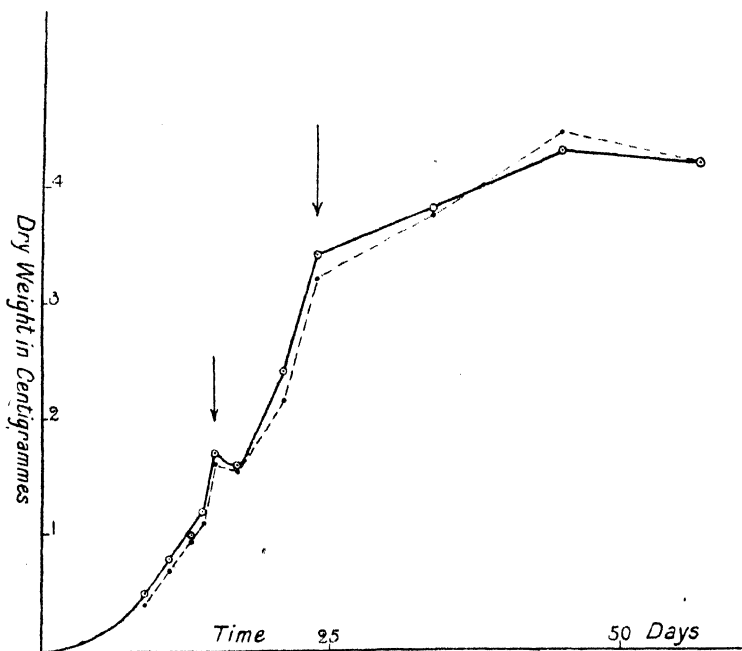


FIG. 2. The growth of roots on *Tradescantia* cuttings (dry weight). The broken line gives wet weight determinations. Arrows show when secondary and tertiary roots appeared.

On October 8 the roots on the heavier set of cuttings had already developed small tertiary roots, but no signs of them were visible in the small set until October 15. Reference to the table and curve shows two points:

1. That the appearance of the tertiary roots in the 2.5 gm. cuttings seems to coincide with a flattening of the S curve (see p. 249).
2. That neither set of roots shows much indication of the development of a third S curve.

The wet weight figures are very irregular, but the dry weight curves suggest a third S curve passing at an early date into a flattened region that continues throughout the winter. Presumably the root production has

reached a balanced proportion in reference to the shoot production, and during the winter months the shoot production has been small, possibly owing to limiting external conditions.

The final weighings given below seem to indicate that when the roots have reached a steady weight the weight bears a rough proportion to the original weight of the cuttings. This correlation is not so well marked as in Loeb's experiments on regeneration in *Bryophyllum* (6 and 7), but his data were obtained in a much shorter time. In these *Tradescantia* experiments the growth over the period of the experiment was slow, and probably time permitted the smaller cuttings to gain in total mass as compared with the larger ones.

TABLE III. *Tradescantia*, Series III.

Number of days' growth.	<i>Tradescantia</i> , 2.5 grm.		<i>Tradescantia</i> , 5.0 grm.	
	Mean weight of wet roots.	Mean weight of dry roots.	Mean weight of wet roots.	Mean weight of dry roots.
	grm.	grm.	grm.	grm.
50	0.569	0.030	0.753	0.039
57	0.677	0.035	0.946	0.049
64	0.714	0.037	0.851	0.046
71	0.647	0.039	0.953	0.055
78	0.547	0.035	0.874	0.055
95	0.612	0.036	0.944	0.056
106	0.655	0.035	0.763	0.054
148	0.618	0.036	0.777	0.056
300	1.357	0.079	1.573	0.095

Experiments with Tomato.

The cuttings, taken from two varieties of tomato, varied much in size, as it was found impossible to accumulate a large enough number of cuttings of equal mass at the start of the experiment. For this reason the figures in the table are given as the *ratio* of the wet or dry weight of the roots to the original weight of the cutting.

The cuttings were grown singly, in inverted flower-pots which stood in dishes of tap-water. There were fifty cuttings of each variety, and five of each were collected at intervals. The experiment was started on July 15, and indications of roots were visible on most of the cuttings by July 20. In the 'Sunrise' series, secondary roots began to appear after eighteen days, but in the 'Princess of Wales' not until after twenty days.

The yield of roots was not so regular from cuttings of great original variability in size, and we have no evidence that root production would show any strict proportion to the original mass of the cutting. The probable errors are not given in the table, but are so large that these results, if they stood alone, would have no value. However, they may be regarded as supplying some indication (when the curves in Figs. 5 and 6 are examined) of the *same* relationship with regard to the rate of growth, and the production of secondary roots, in the case of the tomato as in the case of *Tradescantia*.

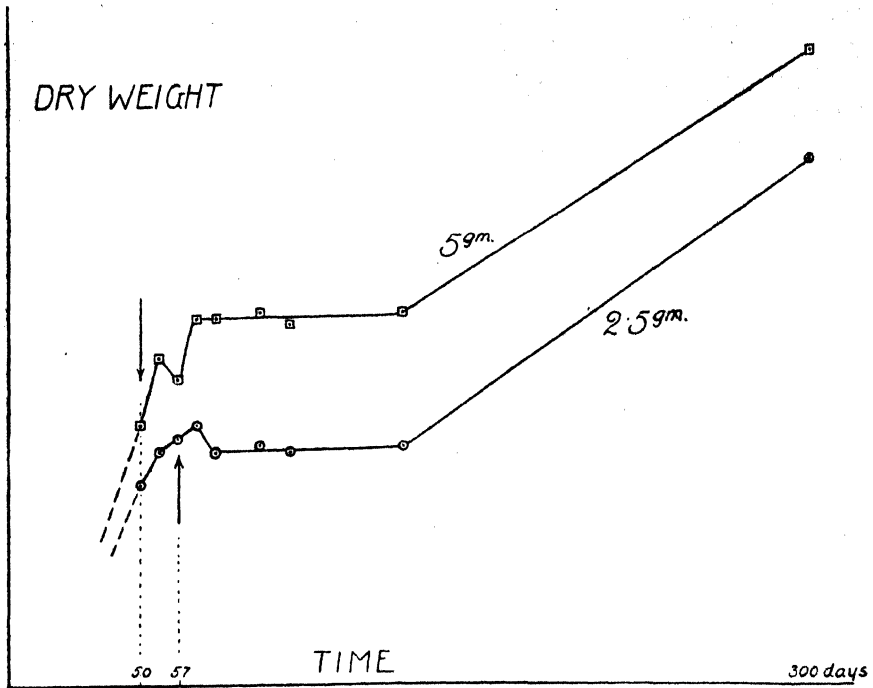


FIG. 3. The production of roots on *Tradescantia* cuttings of initially different weights—5.0 and 2.5 gm. The arrows show when tertiary roots appeared.

TABLE IV. Tomato ('Sunrise').

Number of days' growth.	Mean wet ratio.	Mean dry ratio.
12	0.037	0.0036
13	0.069	0.0053
15	0.045	0.0060
18	0.402	0.28
20	0.327	0.024
22	0.426	0.030
26	0.502	0.039
29	0.619	0.039
32	0.791	0.051
39	0.440	0.038

TABLE V. Tomato ('Princess of Wales').

Number of days' growth.	Mean wet ratio.	Mean dry ratio.
12	0.024	0.0032
13	0.097	0.007
15	0.259	0.025
18	0.418	0.038
20	0.575	0.041
22	0.393	0.031
26	0.578	0.045
29	1.019	0.067
32	1.043	0.066
39	0.633	0.050

4. INTERPRETATION OF EXPERIMENTAL RESULTS.

If the curves obtained in all these series of quantitative observations on root growth are compared, it will be seen that they are of one general type, which we venture to describe as a progressive series of S curves. The S curve is of frequent occurrence, when quantitative data of the progress of growth are recorded, both in the case of animals (Robertson (8)) and of plants (Gregory (4), Schuepp (9), &c.).

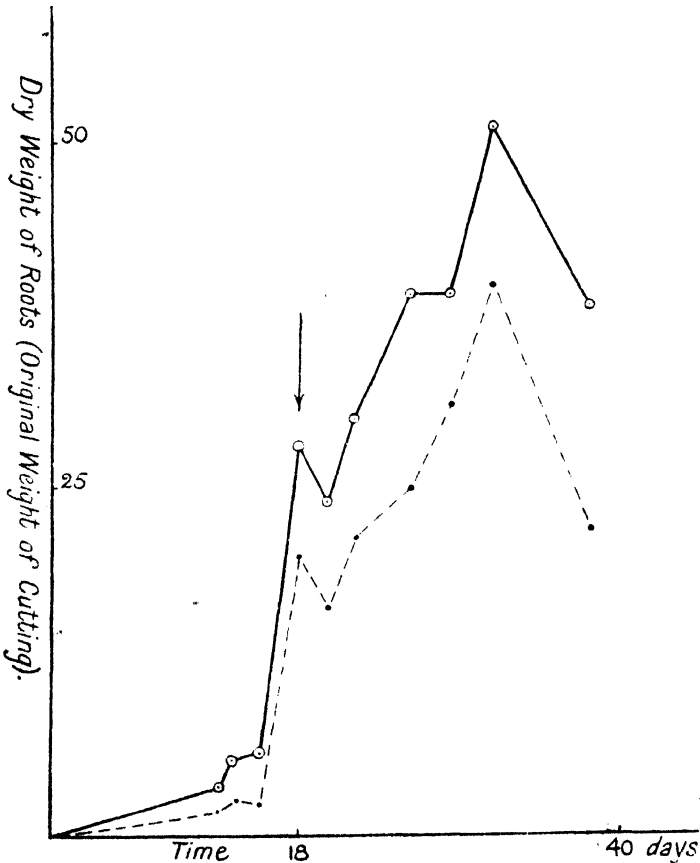


FIG. 4. 'Dry weight ratio' of roots on cuttings of 'Sunrise' tomato (see Table 4 a). The arrow indicates the time of appearance of secondary roots.

A full discussion of this curve will appear in the second paper of this series, so we shall confine ourselves now to two observations which are directly relevant to this special case of the appearance of the S curve in the growth of roots. At the beginning, the cuttings were all rootless, therefore the curve starts at the origin, and indicates an increasing rate of growth which is connected exponentially with time. Subsequently the rate of

growth falls, and a relatively long straight part of the curve indicates a direct proportion between growth and time. Finally the curve is inflected and growth decreases exponentially with time until a brief almost flat portion terminating the first S curve suggests a temporary cessation of growth.

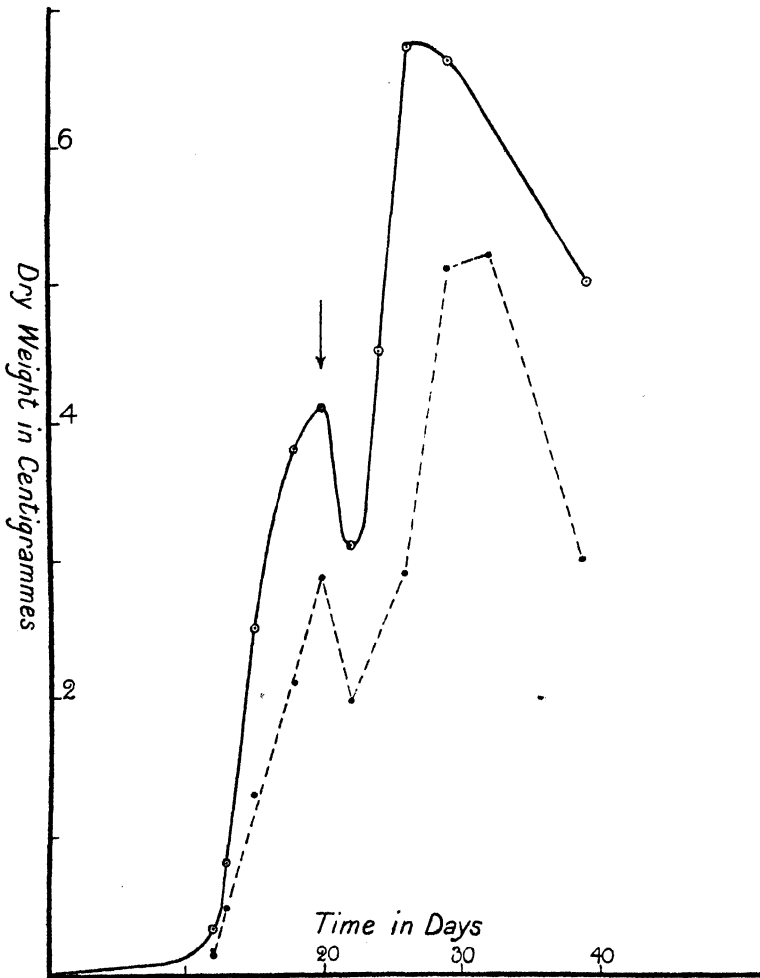


FIG. 5. The dry weight ratio for roots from cuttings of 'Princess of Wales' tomato. The arrow indicates the time of appearance of secondary roots.

We would draw attention to the fact that this last flat region of the curve has obviously some connexion with the development of the lateral roots. In all the curves the point is marked upon the time axis where the occurrence of secondary or tertiary roots was noticed. These points are very definite, especially in the case of *Tradescantia*, where the lateral roots

appear on the 14th day. It will be seen that the termination of the first S curve coincides with the appearance of secondary roots, and the termination of the second S curve with the appearance of tertiary roots (Figs. 1, 2, 5, 6). The theoretical discussion of this phenomenon will be reserved for the second paper of this series.

The second point that emerges is that this S curve, in so far as it applies to roots, is a restatement of the Sachs 'Grand Period' of Growth.

5. THE SACHS 'GRAND PERIOD' OF GROWTH.

Reference to any text-book of plant physiology (e.g. Jost (5), p. 288) will show that this generalization appears to be the complete expression of our knowledge of the quantitative phenomena involved in the growth of roots. It is based up till now entirely upon *length*, and therefore these data are only available for the time *preceding* the appearance of the lateral roots. Hence, the Sachs 'Grand Period' for the growth of roots covers the period represented in our experimental data by the completion of the first S curve. The 'Grand Period' curves have a different shape, because they are usually plotted as *rate curves* (i.e. by plotting changes in length in the time intervals taken). But it is possible to convert the data given in Table I into a rough imitation of a Sachs 'Grand Period', for the first fourteen days (before the secondary roots occur), by calculating the difference in weight between successive readings and reducing this to a relative rate of increase of mass per day. This has been done and the results plotted in Fig. 6. These data are not so suitable for this treatment as the more readily determined increments in length, but comparison of this curve with that of Fig. 1 (which was plotted from the same experimental data) shows, firstly, that the rising section of the 'Grand Period' curve represents the region where the increase in mass is in exponential relation to the time; secondly, that the approximately horizontal region of the Sachs curve represents the straight portion of the curve in Fig. 1; and, thirdly, that the fall in rate at the end of the 'Grand Period' represents that region of the weight curve whose shape we have attributed to the development of the secondary roots.

It is clear, therefore, that, but for the inadequacy of previous methods of measurement, it would have been possible to demonstrate in the growth of roots (at any rate when produced upon cuttings) a series of 'Grand Period' curves. Each one of these represents the period between the origin of one crop of lateral roots and the appearance of a succeeding crop of rootlets arising from this first batch.

The significance of this statement will be considered further in the next paper of this series.

Presumably this type of curve, the S curve or 'Grand Period' curve, will occur until the root mass is in equilibrium with the mass of the leaf and

shoot, when the subsequent curve of growth may be expected to merge into that characteristic of the sum of the activities of the plant.

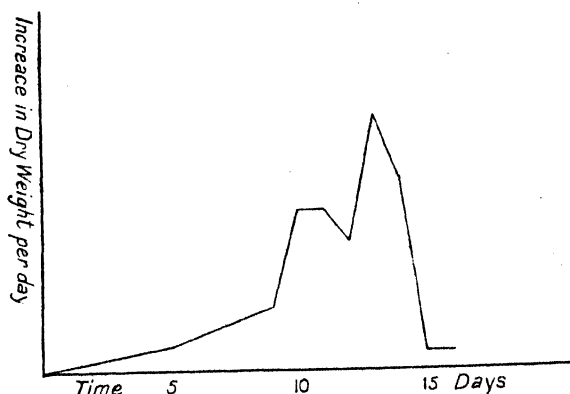


FIG. 6. The data of Table I plotted as a rate-curve showing Sachs's 'Grand Period' of growth. The first part of the curve is suggested.

6. SUMMARY.

1. The data obtained in some quantitative studies of root growth are presented in the form of tables and curves.

2. Roots were chosen, in these experiments, to avoid the more direct influence of the progressive change in photosynthetic area during growth.

3. Cuttings were used, instead of seedlings. In the case of *Tradescantia*, the cuttings were of uniform weight.

4. When plotted, the data provide examples of successive curves of the characteristic S type so frequently found in growth experiments.

5. The time of transition from one S curve to the next is shown to coincide with the time of appearance of a crop of rootlets of subordinate branch order.

6. It is pointed out that a single S curve corresponds to the Sachs 'Grand Period' curve for root growth, and that if his method of measurement had permitted, the rate of growth of roots would have provided a series of such 'Grand Period' curves.

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Growth Studies.

II. An Interpretation of some Growth-curves.

BY

J. H. PRIESTLEY and W. H. PEARSALL.

With four Figures in the Text.

INTRODUCTION.

THE first paper of this series contained the results of a quantitative study of the growth of roots from *Tradescantia* and tomato cuttings. Graphically expressed, these results give a series of S-shaped curves, the initiation of each curve coinciding with the development of a new crop of roots of a subordinate order of branching.

Curves of this type are very familiar in quantitative investigations on growth, when the results are expressed simply as the record of mass, volume, area, or length after different periods of time. This type of curve may therefore be taken as characteristic of many growth reactions. It is proposed to consider a possible explanation of the S curve as exemplified in the growth of yeast and of roots.

No attempt will be made to summarize the extensive literature dealing with the problems of growth. A full discussion of much of the earlier work is given by D'Arcy Thompson (19), and in this paper reference will be made only to facts of interest from the particular point of view here developed. It suffices to point out that the earlier attempts at the explanation of the growth-curve fall into two categories. They are either attempts to find a mathematical formula for the graphical results obtained (Robertson (11, 12, 13), Gregory (7), Schuepp (15, 16)), or else they try to find analogies in curves expressing the relation between other types of reaction and time. The latter method of attack has resulted in the consideration of the analogy between growth and an autocatalytic chemical reaction.

Much profit can be derived from the consideration of the arguments advanced in these first attempts at generalization in a complex field, and as these aspects of the case have been too summarily dismissed by West, Briggs, and Kidd (20), in a recent survey of the literature, it may be useful to outline some of the more important points of interest.

Robertson (14) has contributed some of the most carefully controlled quantitative data yet available for study. He has actively followed both lines of attack outlined above, and has prosecuted both the mathematical analysis of his curves and also the establishment of the analogy between the S curve of growth and the curve for an autocatalytic chemical reaction. His later work shows, however, interesting modifications of his original position.

He begins with the assumption that the autocatalyst, in the successive S curves shown in the growth of certain animals, may be some part of the protoplasmic complex itself, nuclear in the first curve and cytoplasmic in subsequent stages (11, p. 587). Later, he evidently considers that the catalyst must be sought for amongst definite chemical substances, probably provided by the glands responsible for internal secretions known to be associated with growth. This change of position is important, because such a definite catalyst would probably be responsible for modifying the rate of a definite reaction or type of reaction, and such reactions could only form single items in the complex machinery necessary for growth.

Further, the experimental investigation of possible catalysts leads Robertson to a separate consideration of the two regions of different inflexion in the S curve, upon which the same catalyst appears to produce different effects.

A consideration of this change of position appears to us of value as leading to certain preliminary conclusions that seem to be important. Because the quantitative data of growth obtained under uniform external conditions can be represented upon a single curve, it by no means follows that throughout the whole period of growth the same internal factors alone remain operative. It is probably significant that Robertson's continued study of the problem leads him (1) to assume first a greater complexity of internal conditions and (2) to consider separately the different regions of his curves.

In attempting to obtain further insight into the significance of the S growth-curve we have carried the process of analysis somewhat farther, and have found it fruitful to consider this curve as consisting of three distinct regions in which different internal factors are operative. Under these circumstances a mathematical expression for the whole curve seems to have no especial significance.

We propose to consider as examples two cases of plant growth for which data are available—the growth of yeast in a limited supply of nutrient medium and the growth of roots from cuttings as recorded in the first paper of this series. No suggestion is made that the arguments advanced are of general application. Indeed, in the case of the similar curves obtained for the growth of individual leaves (Gregory (7)), it is clear that other factors have there contributed to produce the same result. Probably every case

of the occurrence of an S growth-curve will require individual analysis before profitable generalizations can be developed.

THE RATE OF GROWTH OF YEAST.

It is of interest to note that if all the data available for the growth of yeast are collated, from the time that a few cells are seeded into a definite quantity of suitable nutrient medium until the time that growth comes to a standstill, they give a curve of the general type shown in Fig. 1. Moreover, these data, though appearing in the work of different observers, show that this curve can be divided into three distinct regions, *a*, *b*, and *c*, as shown in Fig. 1. In the region *a* increase in mass is an exponential function of time, in region *b* the rate of growth is directly proportional to time, and in region *c* there is a rapid fall of growth-rate.

The nature of the earlier part of the curve (*a*) was established by Slator (17), who has shown that when the nutrient liquid is inoculated with very small amounts of yeast (1,360 to 90,000 cells per c.c.), a logarithmic rate of increase follows. This may be regarded as the natural rate of increase in

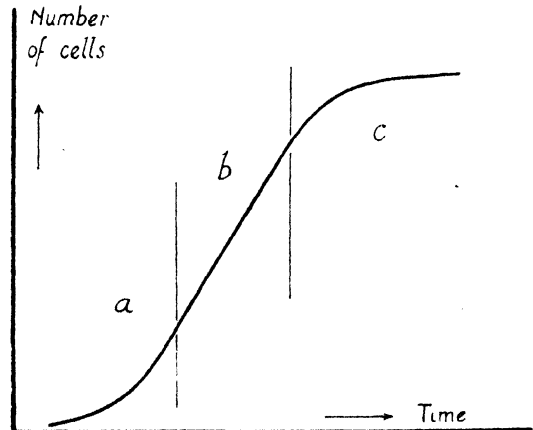


FIG. 1. The growth curve for yeast (diagrammatic).
For explanation see text.

yeast, when every cell is actively growing, and at equal intervals of time one cell gives rise to two, two to four, four to eight, and so on. This exponential rate of increase is even more readily recognized when growth is by fission, as in bacteria.

When the rate of growth becomes proportional to time, Horace Brown (6) supplies valuable evidence showing that the amount of oxygen available is the limiting factor, under the normal conditions of the culture medium. The concentration of oxygen above the nutrient fluid is constant, and hence in this part of the curve the number of cell-divisions in each unit of time also remains constant.

In the last part of the curve, it appears from Adrian Brown's work (5) that the growing crowding of the cells in the nutrient fluid causes the available oxygen supply for an individual cell to fall below the minimum quantity essential for cell-reproduction. This effect increases

with the increase in number of yeast cells, until finally cell-division practically ceases.

It is recognized that this brief statement must necessarily understate the complexity of the conditions prevailing, but it seems so essential that this picture of the mechanism should be clear, that it may be restated with the help of Fig. 2. In this figure successive generations of yeast are pictured as arising at equal intervals of time represented by the horizontal distances.

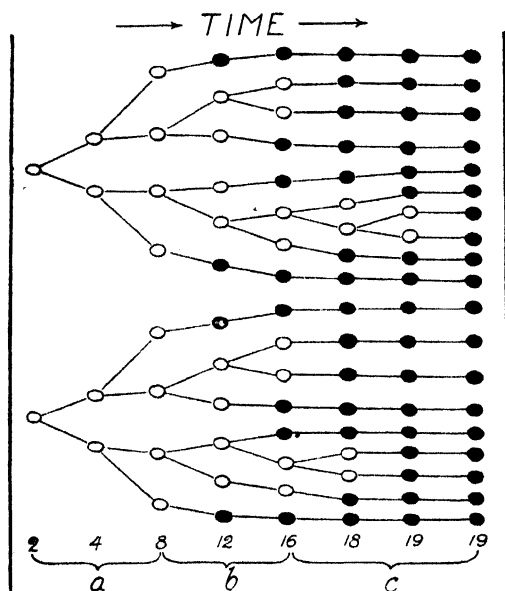


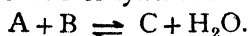
FIG. 2. Diagrammatic representation of the growth of yeast in a limited amount of nutrient solution.

Then in section *a* each cell gives rise to two cells at every time interval. In section *b* a constant number of cells gives rise to new cells, the remaining cells passing over into a resting stage. In region *c* the number of dividing cells decreases until all are in the resting stage and no growth ensues.

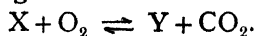
This simple statement neglects the possibility of variation in the rate of growth of individual cells and simply assumes a sharp contrast between the dividing and the resting condition of the cells. It will obviously support further analysis. The transition from the *a* to *b* regions of

the curve is shown to depend upon the limiting supply of oxygen. Growth under aerobic conditions must certainly be based on two general types of reversible reaction that are mutually interdependent:

- (1) the type characteristic of synthetic metabolism



- (2) the type providing for the release of energy



A limiting supply of oxygen will affect the progress of the second type of reaction and hence the rate at which *Y* is formed and energy released. Since we assume synthetic metabolism to depend on the presence of a source of energy, the first type of reaction will also slow down and *C* will be produced more slowly. The reactions of the first type have to be considered as proceeding in a long and complex chain (as in the synthesis of complex proteins from amino-acids), where *C*, the product of one reaction, forms a starting-point for the next reaction in the chain. It is improbable

that the energy relations of all these reactions are the same, and hence, when oxygen supply becomes limiting, all these reactions will not be influenced similarly, and the result will be accumulation of C from some such processes and the slowing down of others for lack of A and B.

The immediate consequence will be local accumulations within the cell, followed possibly by osmotic action and vacuolation or by the appearance of storage products. The general result will be a transition from the active growing condition to the resting stage.

This process of transition to the resting condition occurs most rapidly in the region *c* of the curve, and to judge from a later paper by Slator (18), we may assume that this is in part the result of the accumulation of

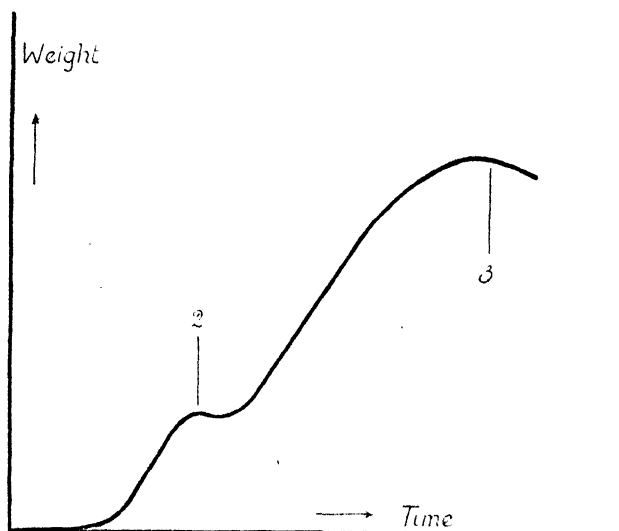


FIG. 3. The increase in weight of roots from cuttings (diagrammatic). Secondary roots appear at 2, tertiary roots appear at 3.

products in some such chemical reactions. These processes are to some extent reversible, for if yeast-cells from a culture that has ceased to grow are transferred to a fresh supply of nutrient medium, a 'lag' period ensues when there is no growth, this being followed by growth at the normal speed. This 'lag' period is presumably the time required for the removal of the accumulation products by diffusion or oxidation.

Undoubtedly this general interpretation of the existing data of the growth of yeast errs on the side of over-simplicity. It gives, however, a mental picture of the causal sequences involved, which forms a useful background for the attempt to interpret the curves obtained for the growth of roots. It has therefore been presented as an introduction to this attempt.

THE GROWTH OF ROOTS.

The growth of roots from cuttings, as shown in the first paper of this series, may be expressed by the curve given in Fig. 3. This curve consists essentially of two successive S curves.

The exponential region of the initial curve appears to represent the period during which a meristem is growing in amount, and in which each meristematic cell divides to give two meristematic cells in a given time interval, as in the *a* region of Fig. 2. Actually the data may show the position of the change later than it really occurs, since what we measure is not merely the mass of the growing meristem but probably to a greater extent the subsequent mass increase due to the differentiation of the cells. This would involve vacuolation (not measured in dry weight determinations), the deposition of thicker walls and membranes, and the inclusion of various accumulation products. There may also be an initial 'lag' period due to the transformation of resting cells in the shoots into meristematic cells, as in the case of yeast resting cells.

The meristem soon ceases to increase exponentially, and the mass increase becomes proportional to time. We have here to seek another factor governing the rate of the reaction. Loeb (9) has shown that the rate of regeneration of roots is directly proportional to the mass of the shoot upon which they are produced. The same influence can be traced in the results of the *Tradescantia* experiments (Priestley and Evershed; see *ante*, pp. 231-2). This suggests that the total mass of meristem produced on the root system will be determined by the supply of root-forming material from the shoot. Under such conditions a constant mass of shoot would maintain the activity of a constant mass of meristem, and the increase in mass of tissue would become proportional to time. In the *b* region of the curve it may therefore be supposed that an equilibrium has been attained between the secretion of root-forming materials by the shoot and their utilization in the root apex. The diffusion gradient of these materials will be approximately constant, and hence in this part of the curve the rate of food supply is assumed to be the factor limiting the rate of growth.

As in Fig. 2, the same number of cells remain actively meristematic in succeeding intervals of time, other cells passing into the resting stage, which in this case means that they proceed to vacuolate and extend in size behind the growing-point.

Since the shoot on which the roots are borne is gradually increasing in size, there should be a slight increase in growth-rate in the later parts of the curve. There is actually, however, a rapid retardation of growth (region *c*), which suggests a falling off in the supply of food to the apical meristem. In the cases examined, this retardation is associated with great regularity with the development of a crop of secondary roots, which

appeared at the same time on all plants in these cultures. The association of this appearance with the horizontal portion of the first S curve seems too close to be accidental, especially as the appearance of tertiary roots produces a similar effect in the second S curve. Tentatively, therefore, it may be supposed that the decreased growth-rate in region *c* of the growth-curve for roots is due to the diversion of food supply to the developing lateral roots. Support is given to this suggestion by the fact that the mass increase per unit of time is the same in the *b* regions of both the first and second S curves—indicating that the rate of food supply has not materially altered.

The objection may then be raised that the mass increment would be the same whether the root-forming material were used at the apical meristem or in the incipient lateral apices. This by no means follows, however, for if the supply of food material is absorbed by the developing lateral meristems, it may well be that initially the greater part of it would be used in oxidation processes involving loss of weight. Such a 'lag' period is noticed by Briggs, Kidd, and West (4) in Kreusler's data. It appears to be shown during the initial development of roots on cuttings, and is known to be a well-marked feature of the growth-curve when yeast in a resting stage is seeded into a nutrient solution (18). The pericycle cells from which lateral roots develop are not actually meristematic, and it seems probable that, as in yeast, a 'lag' stage may occur during their development into meristematic tissue. In addition, if meristematic activity is initiated above the main root apex, the food supply to the apex may temporarily cease and its rate of growth would then decrease, with a resultant decrease in the rate of membrane formation, &c., behind the apex. The flattening of the curve at the time of lateral root formation thus appears to be a very natural effect. While only further experiment can show to what extent the same curve is true for root development in general, we may now utilize the suggestion put forward to consider two further questions:

(1) The rate of growth of roots at high temperatures, as recorded by Leitch (8).

(2) The causal factors in the development of endogenous secondary roots.

THE RATE OF GROWTH OF ROOTS AT HIGH TEMPERATURES.

Leitch (8) has recently given curves for the growth-rate of *Pisum sativum* at temperatures of 30° and 35° C., one of which is reproduced in Fig. 4. Leitch was quite unable to explain these curves, and only recorded them as being the results of repeated experimental confirmation. In the light of the analysis given above, however, it seems possible to put forward an interpretation of these curves.

The rate of growth of roots from seeds must be assumed to depend on two processes: (1) the chemical reactions involved in the meristematic

activity of the growing-point ; (2) the hydrolysis and delivery of growth-promoting substances from the seed reserves. An increase of temperature probably affects the chemical reactions of the growing-point and the hydrolytic reactions in the cotyledons to a similar extent. There would thus be an increased growth-rate and increased rate of formation of food material, but the latter is not formed at the root tip and some time must elapse before it can diffuse to the growing-point from the cotyledons. We should expect to observe, therefore, an initial increase in the meristematic activity of the root tip, due to the temperature effect on the chemical reactions involved. But, as in the work of Blackman and Matthaei (1, 2), it may be assumed that the increase in rate differs in the different processes in

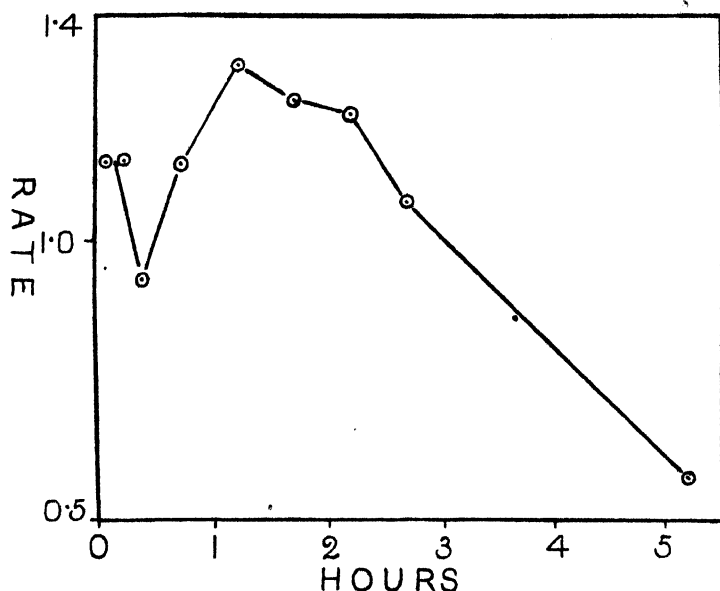


FIG. 4. The growth of pea roots at high temperature ($35^{\circ}\text{C}.$). (After Leitch.)

the growth series, resulting in dislocation of metabolism. The initial increase in growth-rate will be rapidly followed, therefore, by a decrease. An hour or two after the start of the experiment an increased supply of food material becomes available, from the increased rate of hydrolysis in the cotyledons. A secondary temporary increase in growth-rate then appears, until the whole process is slowed down by increasing dislocation of metabolism.

The secondary maximum in growth-rate obtained by Miss Leitch thus falls in line with the point of view advanced here. This secondary maximum may be expected to show a relation to the length of the root at the time measurements are made, and it would seem possible to obtain interesting data as to the rate at which materials move in translocation by a development of this method.

CAUSAL FACTORS IN THE DEVELOPMENT OF ENDOGENOUS
SECONDARY ROOTS.

While the problem of the development of lateral roots cannot yet be fully discussed, it must be considered in the light of evidence hitherto unpublished.

The evidence available supports the view that the supply of sap and of food material for growth is restricted within the endodermal cylinder, which is closed at the apex by the meristematic tissues of the root tip. Within this endodermal cylinder all the cells are vacuolated and differentiated at a fairly early stage, with the exception of the endodermis and the pericycle, which remain relatively embryonic for a much longer time. The fact that they occupy the most favourable position in relation to the balance of supply of food from the conducting elements and of oxygen through the intercellular spaces of the cortex may possibly be the reason for this. The endodermal cylinder is thus lined along the inside with a relatively embryonic tissue, and owing to the normal action of osmosis a considerable hydrostatic sap-pressure also accumulates inside it (Priestley (10)).

Some factor then induces the development of meristematic activity on the part of some of the pericycle cells, usually those opposite the xylem. The exact position of the new activity seems to depend either on the degree of maturation of the pericycle cells or upon the slow accumulation of something diffusing downwards from the shoot, since the lateral roots never approach the root apex closely, but always develop acropetally.

It has been observed that the formation of phellogen below a wound is preceded by a blocking of the exposed surface, a factor presumably leading to increased hydrostatic pressure in the cells beneath the surface, and it has been pointed out also that cork formation is confined to places receiving a free supply of nutrient sap. In addition, the phellogen in roots normally develops inside the endodermis. Both these groups of observations support the suggestion that the supply of nutrient sap and the development of hydrostatic pressure within the endodermis are important factors in initiating the development of the latent activity of the pericycle cells, and in explaining the endogenous origin of lateral roots.

The initiation of this meristematic activity disturbs the gradient of substances diffusing to the main root apex, and as a consequence the growth of the main apex is lessened or stopped, as recorded in our experiments by a fall in the growth-rate. The new lateral meristems in their turn leave behind them vacuolated and differentiated tissues, and the previous growth-rate is thus restored, though apparently not increased, indicating that the total mass increase per unit of time is not dependent upon the amount of meristematic tissue, but upon some other limiting factor, probably in this case the supply of food material.

It is clear that the phenomena so described above would admit of an alternative explanation. If some factor could be found that would inhibit the growth of the main root, then the development of the lateral roots might be ascribed to the accumulation of root-producing substances in the root. So far, however, we have found no adequate cause for the diminution in growth of the main root, and we are therefore inclined to assume that the growth of the main root is temporarily held up by the development of the lateral meristems. The alternative assumption certainly agrees with the case of the fibrous roots of Monocotyledons, where the strong and early development of lateral roots is apparently associated with the inhibition of the meristem of the primary root.

SUMMARY.

1. Data, previously recorded for root growth, provide curves showing a series of successive inflexions of the S type so often obtained in growth experiments.

2. In a brief review of previous work, special attention is given to Robertson's comparison of this type of curve with that of an autocatalytic chemical reaction.

3. It is pointed out that analysis of the physiological conditions involved at different stages of growth is apparently a necessary preliminary in the interpretation of such growth-curves.

4. Such an analysis is attempted for the growth of roots, after previous consideration of the data available for yeast.

5. The early exponential portion of the curve is considered to represent the exponential increase in size of the apical meristem.

6. The mass increase then becomes proportional to time, probably because the supply of root-forming material is delivered at uniform rate.

7. The final decrease in growth is co-ordinated with the initiation of lateral meristems.

8. It is shown that the hypotheses involved give an adequate explanation of the growth of roots at high temperatures (Leitch's data), and permit certain assumptions as to the endogenous origin of lateral roots.

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Studies on Intrafascicular Cambium in Monocotyledons. V.

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With eight Figures in the Text.

IN previous papers in this Journal,¹ I have drawn attention to cases of the occurrence of intrafascicular cambium in a number of monocotyledonous families. Recently the exhaustive researches of Mme Gatin-Allorge² on the anatomy of the peduncle and flower in the Liliiflorae have demonstrated that the occurrence of intrafascicular cambium may be treated as a universal character of the peduncle of the Liliaceae, Iridaceae, and Amaryllidaceae, and that the enlargement of the bundles observed in these families, at the level at which branches are given off to supply the conducting strands of the flower, is due to the activity of this cambium. In 1917 Dauphiné³ published an exquisitely illustrated account of the intrafascicular cambium in *Dracaena*—a tissue whose existence has already been recognized by Fröken Sigrid Andersson,⁴ but which bears no relation to the meristem responsible for the anomalous secondary thickening so well known in the genus. The work of these authors, and that of Lonay on *Ornithogalum*,⁵ Chauveaud on Liliaceous and Amaryllidaceous seedlings,⁶ and Queva on the rhizomatous Uvulariaceae⁷ and the Dioscoreaceae,⁸ in addition to the papers already cited in my previous notes on this subject, make it unnecessary to enlarge further on the existence of intrafascicular cambium in the Liliiflorae; I will now merely mention that I have recently found cambium in the bundles of the very young leaf of *Rhipogonum album*, R. Br., a member of a genus which is not included in Mme Gatin-Allorge's study. But, before leaving the Liliiflorae, there are one or two points in connexion with the bundles of this Cohort to which I should like to refer. I drew attention in 1919⁹ to the fact that in the foliar bundles of a number of Monocotyledons there is a differentiation of the xylem into (i) protoxylem, (ii) primary

¹ Arber, A. (1917), (1918), (1919).

² Gatin, V. C. (1920).

³ Dauphiné, A. (1917).

⁴ Andersson, S. (1888).

⁵ Lonay, H. (1902).

⁶ Chauveaud, G. (1911).

⁷ Queva, C. (1907).

⁸ Queva, C. (1894).

⁹ Arber, A. (1919).

metaxylem, consisting of elements of large lumen, and (iii) smaller elements, which I interpret as arising secondarily from the intrafascicular cambium. I have since noticed this differentiation in two additional cases—*Leucojum aestivum*, L. (Amaryllidaceae) and *Gladiolus* sp. (Iridaceae). In the paper in question I also described and figured certain instances in which the xylem of a lateral branch of a foliar bundle owed its origin exclusively to the secondary xylem of the parent bundle. Recently I have seen a case of this in three other plants—*Crocus carpetanus*, Boiss. et Reut. (Iridaceae), *Asphodelus liburnicus*, Scop. (Liliaceae), and *Aneilema giganteum*, R. Br. (Commelinaceae).

Among the Palms, only two cases of intrafascicular cambium have been hitherto recorded ;¹ to these I can now add three further examples. In serial sections of the shoot apex of *Rhapis humilis*, Blume, the bundles, both in the young leaves and axis, show cambial activity (Fig. 1), and I have also seen the same thing in the plumular leaves of *Chamaerops humilis*, L., and *Areca sapida*, Soland.

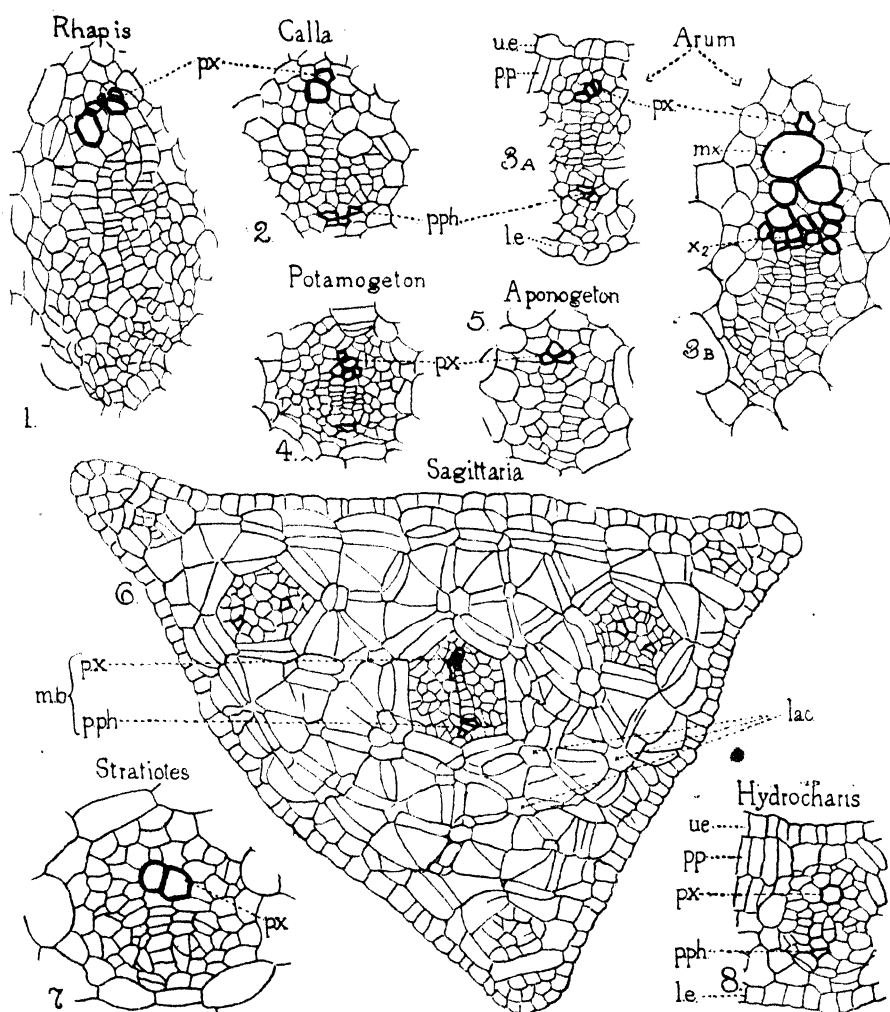
In the case of the Araceae I recorded in 1918² the occurrence of intrafascicular cambium in the rhizome of *Acorus Calamus*, L., and I have since found this tissue in the very young petiole of *Calla palustris*, L. (Fig. 2). In 1914 Lignier³ described and figured cambium in the petiolar bundle of *Arum maculatum*, L., stating, however, that he had examined the mature organ only. His account is somewhat intriguing, since, although he describes the cambium as giving rise to phloem alone, his figure shows a differentiation of the wood into protoxylem, then large-lumened metaxylem, and then xylem elements of smaller calibre, which he also regards as part of the primary wood. I should myself have supposed these latter elements to be secondary, since they recall in arrangement and appearance those small xylem elements which I have shown to be of cambial origin in the leaves of *Anigozanthos*, &c.,⁴ and to which I have again referred in an earlier paragraph of the present paper. In order to try and get further light on the nature of these elements in *Arum*, I have studied microtome sections of apical buds, and hand sections of older leaves of *Arum italicum*, Mill., a species closely related to *A. maculatum*. These sections show that, in the bundles of the petiole and midrib of the very young leaf, there is a radial seriation involving most of the elements between the protoxylem and protophloem. This stage is seen in Fig. 3 A, which was drawn from a leaf so young that the petiole was only about 0.7 mm. in diameter. In the bundles of the midrib and petiole of the mature leaf (Fig. 3 B) the protoxylem is followed by a few large elements (*m.x.*), which I take to be primary metaxylem ; to these again succeed a number of small elements (*x.*₂), whose seriation in radial files, which continue into the

¹ For references see Arber, A. (1917).

³ Lignier, O. (1914).

² Arber, A. (1918).

⁴ Arber, A. (1919).



FIGS. 1-8. (Lettering throughout as follows: *p.x.*, protoxylem; *p.ph.*, protophloem; *p.p.*, palisade parenchyma; *u.e.*, upper epidermis; *l.e.*, lower epidermis.) Fig. 1, *Rhipis humilis*, Blume, bundle from transverse section of stem close to apex ($\times 318$). Fig. 2, *Calla palustris*, L., bundle from transverse section of petiole of very young leaf ($\times 318$). Fig. 3, *Arum italicum*, Mill.; Fig. 3 A, median bundle from transverse section of lamina of very young leaf ($\times 193$); Fig. 3 B, lateral bundle from transverse section of petiole of older leaf ($\times 193$); *m.x.*, primary metaxylem; *x2*, secondary xylem. Fig. 4, *Potamogeton natans*, L., lateral bundle from transverse section of very young petiole ($\times 318$). Fig. 5, *Aponogeton distachyum*, Thunb., principal bundle from transverse section of very young inflorescence axis ($\times 318$). Fig. 6, *Sagittaria sagittifolia*, L., transverse section of very young petiole; *m.b.*, median bundle; *lac.*, lacunae, of which only three are yet visible ($\times 193$). Fig. 7, *Stratiotes aloides*, L., bundle from transverse section of base of very young leaf ($\times 318$). Fig. 8, *Hydrocharis morsus-ranae*, L., median bundle from transverse section of limb of very young leaf ($\times 318$).

secondary phloem, seem to me to make it impossible to doubt their origin from the intrafascicular cambium. From my sections and from the evidence of Lignier's own figure, I thus conclude that the intrafascicular cambium in the *Arum* leaf gives rise not only to phloem, but also to a certain amount of secondary xylem.

In searching for intrafascicular cambium in Monocotyledons, the chief difficulty arises in connexion with those families that are mainly aquatic, and in which the vascular skeleton is correspondingly reduced. The poor development of the bundles in many of the representatives of such families probably accounts for the fact that the existing records of intrafascicular cambium in the Helobieae are apparently confined to Andersson's discovery of this tissue in *Triglochin*,¹ and my note on the existence of 'very slight and irregular cambial activity' in the leaves of two species of *Potamogeton*.² But I have found, on re-examining the latter genus in greater detail, that the cambium need not be described in such qualified terms. Microtome sections of apical buds of *Potamogeton natans*, L., reveal the existence, in the foliar bundles, of a well-marked seriation of the elements between the protoxylem and protophloem (Fig. 4). In very young stages the seriation may involve only one or two files of cells, but at later stages it becomes more extensive.

There has been no record hitherto of the occurrence of cambium in the Alismaceae, Aponogetonaceae, or Hydrocharitaceae, but I have recently found that, by cutting microtome series through the shoot apices, and thus exposing the vascular bundles in their initial phases, the existence of this tissue can be demonstrated in these families also. The petiole of *Sagittaria sagittifolia*, L. (Alismaceae), represented in Fig. 6, was so young that it was only about 0.5 mm. in width, and the lacunae (*lac.*), that would have eventually given a lace-like appearance to the transverse section, were only in two or three cases making their appearance. Cambium occurs in the median bundle at this stage, but it is inconspicuous, since only one or two files of cells extending between the protoxylem (*p.px.*) and protophloem (*p.ph.*) are radially arranged. The bundle in this phase very closely recalls Queva's description of the median vascular strand of the very young leaf in the Liliaceous genera *Uvularia* and *Tricyrtis*.³ The cambial activity in *Sagittaria* is extremely ephemeral, and one may search in vain for any sign of it in older petioles.

On cutting serial sections through the apical bud of *Aponogeton distachyum*, Thunb. (Aponogetonaceae), I found that there is a distinct development of intrafascicular cambium in the rudimentary inflorescence axis; that to which the bundle drawn in Fig. 5 belonged, was so young as to be only 0.3 mm. in diameter.

In the case of the Hydrocharitaceae, I examined serial sections through

¹ Andersson, S. (1888).

² Arber, A. (1918).

³ Queva, C. (1907).

the shoot apex of *Hydrocharis Morsus-ranae*, L., and *Stratiotes aloides*, L., and in both these plants I found traces of intrafascicular cambium, though on an inconspicuous scale (Figs. 8 and 7).

SUMMARY.

This paper forms a continuation of my previous notes on the same subject in this Journal for 1917, 1918, and 1919. Further references in the literature to intrafascicular cambium among Monocotyledons are considered, and certain new cases are recorded.

It is shown that Lignier was probably mistaken in attributing only phloem-forming activity to the cambium in the petiolar bundle of *Arum maculatum*, since, in the corresponding bundles of the closely related *A. italicum*, secondary xylem is found to be also formed—while Lignier's own figure leaves little doubt that the same process takes place in *A. maculatum*.

Special attention is paid in the present paper to the search for cambium in the slightly developed vascular bundles of the Helobieae, and it is shown that the Alismaceae, Aponogetonaceae, and Hydrocharitaceae can now be added to the list of monocotyledonous families in some member of which this tissue has been observed—an addition which brings the number of such families to twenty-two.

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CAMBRIDGE.

LISTS OF MEMOIRS CITED.

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On the Germination and Growth of Fungi at various Temperatures and in various Concentrations of Oxygen and of Carbon Dioxide.

BY

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With four Figures in the Text.

INTRODUCTORY.

THE present investigation was carried out under the auspices of the Food Investigation Board of the Scientific and Industrial Research Department, and had for its object the examination of the behaviour of fungi under the conditions prevailing in the practice of fruit storage. There are two methods of storage, known respectively as 'cold storage' and 'gas storage', the former of which is now widely established in practice, while the latter is still in an experimental stage. As the scope of this investigation was to a large extent determined by considerations of a practical nature, a brief account of these two methods may appropriately be given here.

In the ordinary method of cold-storing fruits, where the fruit is subsequently to be used for general household purposes (and not, for example, for wholesale jam manufacture), a temperature of less than 0°C . or thereby is not admissible. In actual practice a temperature of $2-3^{\circ}\text{C}$. is the lowest usually considered safe. No attempt was therefore made to test the behaviour of fungi at temperatures below this limit, and in fact, except for a few experiments, a temperature of 5°C . was the lowest employed.

The gas storage method is based on the retarding action of carbon dioxide on metabolic processes, so that the retarding effect of a lowering of temperature in ordinary cold storage is replaced or augmented by that due to a certain concentration of carbon dioxide in the atmosphere of the store. In practice the carbon dioxide is derived from the respiration of the fruit itself (the concentration being maintained at a steady level by special devices). In view of the fact that the concentration of oxygen in

the atmosphere of the store must not fall so low that anaerobic respiration sets in, it follows that the maximum concentration of carbon dioxide available in practice is something under 20 per cent. In the experiments to be described here the effects of much higher concentrations of carbon dioxide on fungal growth were tested, but special attention was directed to the effects of the lower concentrations admissible in practice.

HISTORICAL.

Numerous statements occur sporadically throughout fungal literature in connexion with the effect of *temperature* on fungal growth. Only the more recent and important papers dealing specially with fungi causing fruit-rot call for mention here.

Schneider-Orelli (1) cites the following fungi as producing fruit-rot: *Penicillium glaucum*, *P. italicum*, *Botrytis cinerea*, *Monilia fructigena*, *Gloeosporium fructigenum*, *G. album*, *Fusarium putrefaciens*, *Cladosporium herbarum*, *Mucor piriformis*, and *Rhizopus nigricans*. He states that with the exception of *P. italicum*, which is the most sensitive of all to low temperature, all the preceding are capable of growth at 4.5° C., and the majority are even capable of slow growth in the neighbourhood of 0° C. The most active parasites are *Botrytis cinerea* and *Monilia fructigena*, and the former does more damage in cold store by reason of its greater capacity for growth at low temperature. *Monilia* tends to disappear from the store as time goes on. The explanation put forward for this is that the low temperature, though not entirely inhibiting the growth of the fungus, is effective in preventing its spore development, in which respect the absence of light is probably an important contributory factor.

Both these fungi, *Botrytis cinerea* and *Monilia fructigena*, are, according to this observer, of special importance in that they are able to attack a great variety of fruits over a wide range in respect of ripeness and over a wide range of temperature. The other fungi listed have more limited parasitic powers. Thus they may only be able to bring about attack when the fruit has reached an advanced stage of ripeness. Of this type is *Penicillium glaucum*, which shows markedly selective action in accordance with the ripeness of the fruit, and which, according to this author, can only attack the late-ripening varieties of home fruits with difficulty, at least for a long time. Its abundant spore-formation and its capacity to attack ripe fruit make it an important parasite during the late period of storage.

Ames (2) determined the cardinal points for temperature (minimum, optimum, maximum) of a number of fungi causing fruit-rot. The minimum temperatures for germination given by this observer are as follows: for *Monilia fructigena* and *Penicillium digitatum*, 1°; for *Rhizopus nigricans*

and *Glomerella rufomaculans*, 3°; for *Thielaviopsis paradoxa*, 4-5°; and for *Cephalothecium roseum*, 6-9°. The minimum temperature for fructification is in all cases several degrees higher than that for growth.

Edgerton (3) determined the relation of growth to temperature of thirty strains of *Glomerella*. This paper is interesting as illustrating the amount of variation as regards response to different temperatures shown by organisms which are morphologically very similar to each other.

Brooks and Cooley (4) examined in detail the temperature relationships of twelve fungi which produce rot of apple in storage. For tables and graphs of the rate of growth of the various fungi on artificial medium and on apple, the original paper should be consulted. The following results may be quoted here:

1. Spores of *Alternaria* sp., *Botrytis cinerea*, *Penicillium expansum*, and *Sclerotinia cinerea* germinated slowly at 0° on corn-meal agar medium; spores of *Cephalothecium roseum*, *Fusarium radiculicola*, *Glomerella cingulata*, and *Pestalozzia funerea* failed to germinate at 0°, but slowly germinated at 5°; spores of *Aspergillus niger* failed to germinate at 10°.

2. The minimum temperature for the growth of some fungi in the case of inoculations on fruit is higher than that for growth on artificial medium, and in general the inhibitory effect of low temperature on the early phases of growth is more pronounced in the case of inoculations on fruit than for inoculations on artificial medium. Again, in the case of the weaker parasites, the minimum for growth on the fruit is dependent upon the state of maturity of the latter, being lower according as the fruit is in a more advanced stage of ripeness.

3. In the case of *Penicillium expansum* it was noted that the fungus, after starting at ordinary temperatures, was able to continue growth at 0°, whereas it could not initiate attack at the latter temperature. The authors therefore point out the advisability of immediate storage.

In connexion with the last-mentioned point it is interesting to note that Kirchner (5) records a similar phenomenon for the growth of the roots of seedlings.

The effect on growth of *atmospheric composition* has been studied on a large variety of plants and plant parts. The two factors of importance in the present connexion are: (1) the influence of varied concentration of carbon dioxide, (2) the influence of varied concentration of oxygen.

Carbon dioxide. That a concentration of carbon dioxide greater than that normally present in the atmosphere accelerates the growth of higher plants has been claimed by some workers, e. g. by Demoussy (6), Fischer (7), Chapin (8), and Kisselew (9). Brown and Escombe (10), however, failed to observe any appreciable increase of yield brought about by increased concentrations of carbon dioxide, but on the other hand recorded considerable morphological effects produced in this way (see on this point

Farmer and Chandler (11)).¹ In the case of green plants, though Chapin considers carbon dioxide to act here mainly as a stimulant merely, the effect is complicated by its use for photosynthetic purposes, and on that account these results are scarcely relevant to the present investigation.

Fränkel (12) examined the effect of carbon dioxide on the growth of various micro-organisms on liquid or solid culture media. He found that whereas some organisms, e. g. the true beer yeasts, can grow as well in an atmosphere of 100 per cent. CO_2 as in air, the vast majority both of saprophytic and parasitic forms are inhibited by such treatment. He showed that this inhibition was due to the actual presence of carbon dioxide and not to mere absence of oxygen, and that the effect was one of inhibition and not of killing, as the organisms subsequently grew normally when again placed in ordinary air. He also pointed out the marked variations in susceptibility of different individuals in the same culture (quite apart from spore forms), a feature which was also emphasized by Frankland (13).

Lopriore (14), who gives a full account of the earlier literature, showed among other things that the germination of the spores of *Mucor mucedo* was slowed by the action of 10 per cent. CO_2 in the atmosphere, and that undiluted carbon dioxide, though producing total inhibition, did not kill the spores even after an exposure of three months. Sporangium formation was more readily suppressed than spore germination. Lopriore noted a tendency of the mycelium to form swollen cells under the carbon dioxide treatment. He also states that low percentages of CO_2 (1–10 per cent.) accelerate the growth of pollen-tubes.

According to Plummer (15) the ammonifying bacteria are very insensitive to the action of carbon dioxide, 60 per cent. of this gas in the atmosphere having no appreciable effect on the rate of ammonification.

Kidd (16), working with seeds of *Brassica alba*, found that the germination of the latter was inhibited by comparatively low concentrations (10 per cent.) of carbon dioxide. The retarding effect of a given concentration of carbon dioxide was dependent upon the pressure of oxygen present, being greater the lower the oxygen pressure. Kidd also describes acceleration of growth caused by a sufficiently low concentration of carbon dioxide.

Oxygen. Out of the very voluminous literature dealing with the effect of oxygen pressure on plant growth, it is only necessary here to cite the work of Porodko (17), in which maximal and minimal concentrations of oxygen for a number of micro-organisms are given. From this work it appears that aerobic organisms are very insensitive to changes in oxygen pressure. Thus for *Penicillium glaucum* the maximum pressure at which

¹ It is, however, possible that some of the effects recorded may have been due to impurities in the carbon dioxide employed.

the fungus will grow is as high as 3.5 atmospheres, while the minimal pressure is well below 1 per cent. of an atmosphere. In some cases the minimal oxygen pressure is extraordinarily low, as for example 0.00016 per cent. for *Bacillus subtilis*. While obligate anaerobes have a very low oxygen maximum (0.003 per cent. for *Clostridium butyricum*), facultative anaerobes were found by Porodko to be as resistant to increased oxygen pressures as obligate aerobes, and in some cases more so.

The general result of the work just cited, which is in agreement with earlier work on this subject, was to indicate that variations in the oxygen pressure would be found to be of little importance in connexion with the purposes of the present investigation.

Finally, mention may be made of the work of Berghaus (18) and of Chlopin and Tammann (19), dealing with the effect of varying gas-pressure upon the growth of micro-organisms. According to these investigators the effect of mere varying pressure of a neutral gas (hydrogen or nitrogen) upon the growth of micro-organisms is negligible within very wide limits.

EXPERIMENTAL METHOD.

The various temperature conditions were obtained by means of ordinary Hearson incubators in the case of the higher temperatures (15–25°), and for lower temperatures (2–10°) an automatically regulated small 'Isko' refrigerator was used. The temperature of the latter was controlled electrically by means of a toluene regulator and a relay which stopped and started a pump circulating sulphur dioxide. It was constant to within half a degree on either side of the mean value.

As regards the setting up of fungal cultures in the various atmospheres, the technique described by Kidd (16, p. 411) was followed throughout. Large glass containers of 3½–5 litres capacity (of the type employed as desiccators) were used as receptacles for the fungal cultures. The cultures intended for study, together with a little water to check evaporation, were placed in these, the lids luted down with 'resin cerate', and the required concentration of carbon dioxide in the internal atmosphere made up after the manner described by Kidd—that is, the containers were attached to an air-pump, the enclosed air exhausted to the necessary amount as measured by a manometer (7.5 cm. Hg vacuum for 10 per cent., 15 cm. for 20 per cent., &c.), and the pressure again brought back to normal by the introduction of carbon dioxide gas from a cylinder. The latter gas on analysis gave 98 per cent. purity, the remaining 2 per cent. not being absorbable in either alkali or in alkaline pyrogallol, and therefore being presumably nitrogen.

The composition of the atmosphere in the containers was systematically tested and checked by analysis. For this purpose a large size of Haldane gas apparatus was used. By this means the percentage of carbon dioxide

is readily determined to within 0.1 per cent. With care, much greater accuracy is possible, but this was rarely required. From numerous analyses it was found that with ordinary care the carbon dioxide content of the experimental atmospheres as set up in the manner above described never varied by more than $\frac{1}{2}$ per cent. from the value intended.

Determinations of the oxygen content were only made occasionally, and were carried out by means of the same apparatus.

The following is a list of the fungi used at one time or other in the course of the present investigation, together with a statement of the source from which they were derived:

<i>Botrytis cinerea</i>	Originally obtained from Centralbureau voor Schimmelcultures, Holland.
<i>B. parasitica.</i>	Isolated from onion.
<i>Aspergillus repens</i>	All stock laboratory cultures.
<i>Mucor</i> sp.	
<i>Rhizopus nigricans</i>	
<i>Penicillium glaucum</i>	
<i>Monilia cinerea</i>	All isolated from rotted apples.
<i>Fusarium</i> sp.	
<i>Phoma roseola</i>	
<i>Alternaria grossulariae</i> ¹	
<i>Sphaeropsis malorum</i>	

and a *Botrytis cinerea*, also obtained from apple, and which appeared to be identical with the other culture of this organism.

Stock cultures of these fungi were grown at laboratory temperature in diffuse light on sloped tubes of potato agar.

Experiments dealing with the effect on Germination of various concentrations of Carbon Dioxide and Oxygen.

The general nature of the effects produced was worked out in the first instance with spores of *Botrytis cinerea*. A series of experiments was carried out showing the relation between the amount of germination and the following three factors: (1) concentration of carbon dioxide in the atmosphere; (2) density of spore suspension; (3) concentration of nutrient.

The effect on germination (determined quantitatively as percentage of spores germinating or as the average length of the germ-tube produced) of the second and third of the factors mentioned was known from earlier work to be as follows. The amount of germination in a given time increases with concentration of the nutrient up to a certain limit.² The amount of germination in a given nutrient decreases with increasing density of spore

¹ Identified as such by Horne. Ann. Appl. Biol., vii. 190, foot-note 3, 1920.

² Cf. Brown, this volume, p. 108.

suspension; this effect is most strikingly shown in the case of lower concentrations of nutrient.

In the following tables (I-III) the nutrient employed was a turnip extract obtained by extracting the juice of turnips boiled without any addition of water. This full-strength extract is denoted by T.E., and the various dilutions with water as T.E./10, &c.

The different densities of spore suspension are denoted by m, m/10, m/100, where m denotes a suspension containing 0.1 c.c. of wet spores in 10 c.c. liquid. In order to obtain these suspensions, spores were washed from a plate culture of *Botrytis* after the manner described in an earlier paper¹ and freed from mycelium by filtering through muslin. The spore suspension was then centrifugalized about six times in changes of water in order to complete washing, then finally centrifugalized in a graduated tube and the volume of spores determined. This being known, the various dilutions in the various concentrations of nutrient could be made up.

The germination tests were carried out as follows: Drops of the various suspensions were placed on clean glass slides, the latter placed on a rack in a moist container, and the required atmospheric conditions set up as already described.

These tests were carried out at ordinary laboratory temperature (15-18°), the various containers being placed side by side, so that they were under similar temperature conditions throughout. From the method of preparing the spore suspensions complete absence of contamination could not be guaranteed, but as the tests were of short duration, any accidental slight contamination was of no importance.

TABLE I.

Botrytis cinerea.

Percentage of Germinated Spores after 20 Hours' Germination (based on at least 100 counts in each case).

Spore Density.	Nutrient.	Air.	10 % CO ₂ .	20 % CO ₂ .	30 % CO ₂ .
m/100	H ₂ O	37	5	0	0
	T.E./10,000	72	47	0	0
	T.E./1,000	90-100	75	9	0
	T.E./100	90-100	74	25	0
m/10	H ₂ O	0	0	0	0
	T.E./10,000	3	0	0	0
	T.E./1,000	51	25	2	0
	T.E./100	80	63	18	0
m	H ₂ O	0	0	0	0
	T.E./10,000	0	0	0	0
	T.E./1,000	0	0	0	0
	T.E./100	36	23	0	0
	T.E./10	84	64	8	0

Table II gives the average length of germ-tube in the same experiment, this quantity being determined by measuring by means of a micrometer the lengths of all the germ-tubes shown by the counted spores, and dividing

¹ W. Brown: Ann. Bot., xxix. 319, 1915.

the total length by the total number of spores counted. This quantity gives a better representation of the picture presented than does a mere count of the percentage of germinated spores.

TABLE II.

*Botrytis cinerea.**Average Length of Germ-tube after 20 Hours' Germination.*

Spore Density.	Nutrient.	Air.	10 % CO ₂ .	20 % CO ₂ .	30 % CO ₂ .
m/100	H ₂ O	0.6	0.04	0	0
	T.E./10,000	1.45	1.0	0	0
	T.E./1,000	4.0	2.5	0.1	0
	T.E./100	6.8	3.3	0.25	0
m/10	H ₂ O	0	0	0	0
	T.E./10,000	0.05	0	0	0
	T.E./1,000	1.16	0.45	0.01	0
	T.E./100	5.0	2.6	0.25	0
m	H ₂ O	0	0	0	0
	T.E./10,000	0	0	0	0
	T.E./1,000	0	0	0	0
	T.E./100	0.52	0.5	0	0
	T.E./10	3.35	2.50	0.15	0

We see from the above tables that, within the limits of nutrient, density of spore suspension, and concentration of carbon dioxide employed, the following factors act in retardation of germination: (1) dilution of nutrient, (2) increase in density of spore suspension, (3) increased concentration of carbon dioxide. The effect of these various factors is more strongly shown in Table II than in Table I. Thus, to take a particular instance:

With m/100 spore suspension and in T.E./100 nutrient, the effect of 20 per cent. CO₂ as compared with air is to reduce the percentage of germination from about 100 to 25, i.e. to one-fourth; whereas the corresponding effect on the average germ-tube length is from 6.8 to 0.25, i.e. to one-twentyseventh. This is, of course, due to the fact, that the smaller percentage of spores which have germinated in 20 per cent. CO₂ have on the average much shorter germ-tubes than have the corresponding ones in air.

On comparing the suspensions of spores in water with those in the various turnip extracts, it is seen that the relative retarding effect of a given concentration of carbon dioxide is greater in the former case. The magnitude of this differential effect depends on the time at which the measurements are made and tends to become less as time goes on. Nevertheless, that the effect persists even after long time will be seen later, when it is shown that inhibition of germination of *Botrytis* spores is produced by 30 per cent. CO₂ when the spores are sown in water, whereas a concentration of somewhat over 50 per cent. is required to stop germination of the spores when they are sown in nutrient.

The retarding effect of density of spore suspension on germination is theoretically interesting, though in regard to infection by the spores it is

probably of no practical importance, seeing that such a density of suspension as that represented by m is very much greater than any that would ever be met with in practice. For densities such as $m/100$ and less, this mass effect arising from the spores becomes less pronounced, and for the dilute suspensions which are typically used for germination and infection studies its effect is more or less negligible.

In the foregoing experiments the concentration of oxygen was not kept absolutely constant in the different cases. Taking ordinary air as containing 20 per cent. oxygen, the concentration of the latter in the container set up with 10 per cent. CO_2 would be approximately 18 per cent. ($20 \text{ per cent.} \times 90 \div 100$), and the corresponding oxygen concentrations for the experiments with 20 per cent. and 30 per cent. CO_2 would be 16 per cent. and 14 per cent. respectively. The following tables show that such variations of oxygen concentration are of negligible importance, and, more generally, that the germination of *Botrytis* spores is independent of oxygen concentration over a wide range.

The various oxygen concentrations were made up by suitable admixture of atmospheric air and of oxygen or nitrogen from cylinders. The method of procedure was that employed for the carbon dioxide atmospheres. The symbols used in the following table are the same as in the preceding tables.

TABLE III.

*Effect of Oxygen on Germination of Botrytis Spores.*A. *Effect of Increased Oxygen.*

% Oxygen.	% Germination of spores in water.		
	$m/100.$	$m/10.$	$m.$
21	75	59	30
30	95	47	30
50	45	23	0
80	45	25	0

B. *Effect of Decreased Oxygen.*

% Oxygen.	Spores in water.			Spores in T.E./10.		
	$m/100.$	$m/10.$	$m.$	$m/100.$	$m/10.$	$m.$
21	15	3	0	83	79	20
5	20	4	0	84	70	20
1	0	0	0	20	—	5

It will be observed that the amount of germination in a given time diminishes with increasing concentration of oxygen, but only very slowly, so that even in water quite a good germination results in 80 per cent. oxygen. Again, as the oxygen pressure is diminished, no appreciable effect is shown until very low concentrations of oxygen (*c.* 1 per cent.) are reached. These results were in accordance with anticipation based on the statements of previous workers already cited, and they showed that, as far as the present investigation was concerned, the effects of changes of oxygen pressure on spore germination could be ignored. In subsequent experiments dealing with the rate of growth of fungal colonies this point was

again examined, as it was thought conceivable that a diminution of oxygen pressure which would not affect the germination of spores might affect the rate of growth of a large fungal colony. Experiments were carried out to compare the growth of *Sphaeropsis* colonies in containers in air at atmospheric pressure with that of similar colonies maintained in air at 40 per cent. of the normal pressure. No definite difference could be established between the former growing at 20 per cent. oxygen pressure and the latter at 8 per cent. It was therefore concluded that, for all the purposes of the present investigation, oxygen pressure was a factor of no importance within the limits laid down by the practical aspects of the problem. As regards the effect of the composition of the atmosphere on the germination and growth of fungi, attention was therefore directed solely to the carbon dioxide constituent.

The general nature of the retarding action of carbon dioxide on germination having been studied as above described, experiments were now set up to determine what concentrations of carbon dioxide were necessary to inhibit the germination of fungal spores under various conditions of nutrition. Parallel series of germination tests were set up, in the one case in water and in the other in a good nutrient. For the latter a turnip extract of one-fifth full strength was used. These experiments were carried out at laboratory temperature.

The general type of result will be seen from the following table. The various preparations were set up in the usual way in moist containers. The table describes the progress which has been made in germination after seven days from the start of the experiment.

TABLE IV.
Spores sown in T.E./5.

<i>Fungus.</i>	20 % CO ₂	30 %.	40 %.	50 %.	60 %.
<i>Botrytis parasitica</i>	weft	weft	strong germination	c. 50 % germination	Very occasional papilla
<i>B. cinerea</i> . . .	"	"	good germination	none	none
<i>Monilia cinerea</i> .	"	"	"	rare germination	none
<i>Mucor</i> sp. . . .	long germ- tubes	short germ- tubes	none	none	none
<i>Rhizopus nigricans</i>	weft	weft	weft	good germination	rare germination

Penicillium glaucum and *Fusarium* sp. were the only two fungi which were found to germinate strongly in 60 per cent. CO₂.

It must be observed that the time of seven days allowed for germination is purely arbitrary, and that though in some cases germination had not taken place in this time, it does not follow that some germination would not eventually have occurred.

In all cases it was observed that the carbon dioxide treatment did not appear to harm the fungi in any way, for they germinated normally and

with more or less their usual vigour when again brought into normal atmospheric conditions. Thus the question whether a given concentration of carbon dioxide produced absolute inhibition could only be determined by experiments lasting a very long time. The incubation time for germination in a good nutrient in the neighbourhood of 18° varies for the different fungi, being as low as 4-6 hours for normal *Botrytis* spores and as much as about one day for spores of *Aspergillus repens*. For most of the fungi used the time required for germination in air at ordinary laboratory temperature is about eight hours, and thus the period of seven days chosen in the above experiment is about 20 times the normal incubation period for germination. A fungus, therefore, which fails to germinate under the conditions of the experiment in a period 20 times its normal germination period may be looked upon as completely inhibited to all intents and purposes.

In carrying out these observations, an interesting behaviour was noticed in the case of germ-tubes of *Rhizopus nigricans*. In 50 per cent. CO₂ spores of this fungus showed universal germination, but the germ-tubes had a peculiar appearance. Instead of the normal long straight cylindrical hyphae of uniform diameter the germ-tubes here appeared as short stunted structures of very irregular shape. In optical section they presented a very ragged outline, and their diameter, though varying widely in different parts, was much in excess of the normal. The same appearance was observed in the germ-tubes in 60 per cent. CO₂, but not in those in 40 per cent. These germ-tubes were strongly reminiscent of the cell swellings in species of Mucorineae described by various workers (Ritter, Wehmer)¹ as 'giant cells' (*Riesenzellen*). The latter have been shown to be due to the accumulation of excess of acid in the culture solution, and it appears probable that the formative factor in the present case is acidity due to the high pressure of carbon dioxide. On removal of these distorted germ-tubes to ordinary air, they at once continued their growth in the normal fashion.

The following table gives the concentrations of carbon dioxide which were found to stop germination at 15-18° over a period of seven days:

TABLE V.

Fungus.	Spores sown in T.E. 5.	Spores sown in water.
<i>Aspergillus repens</i>	40	—
<i>Mucor</i> sp.	40	—
<i>Botrytis cinerea</i> (both strains)	50 +	20-30
<i>Monilia cinerea</i>	50 +	20
<i>Phoma roseola</i>	50 +	20
<i>Alternaria grossulariae</i> . .	50-60	> 30
<i>Rhizopus nigricans</i>	60	20
<i>Botrytis parasitica</i>	60 +	20
<i>Sphaeropsis malorum</i> . . .	60-70	30
<i>Fusarium</i> sp.	80 +	30
<i>Penicillium glaucum</i>	80-95	> 30

¹ See on this point Ritter: Jahrb. f. wiss. Bot., lii. 351-403, 1913. Wehmer: Ber. deut. bot. Gesell., xxxi. 257-68, 1913. Lopriore: l. c.

Of all the fungi tested, *Penicillium glaucum* is the most insensitive to carbon dioxide. When we remember that, in the case of an atmosphere which gave 95 per cent. CO_2 on analysis, the bulk of the remaining 5 per cent. is nitrogen, it follows that limited supply of oxygen was now probably entering in as a factor. It is fairly doubtful, therefore, if *Penicillium glaucum* can be inhibited entirely by carbon dioxide alone—that is, within the limits of one atmosphere pressure. This again illustrates the very marked resistance of this organism to external conditions.

The effect of nutrient in relation to the inhibition of germination by carbon dioxide is again shown in the above table.

Speaking generally, we may say that the concentration of carbon dioxide which inhibits germination of fungal spores at ordinary temperatures lies in the neighbourhood of 20–30 per cent. when the spores are sown in water, and in the neighbourhood of 50–60 per cent. when the spores are sown in a good nutrient. These concentrations are not admissible in the practice of the gas storage method, and therefore inhibition of germination of the fungal spores cannot be realized under practical conditions. It remains to examine the degree of retardation which can be effected by concentrations which do not exceed 20 per cent., and especially to determine what effect as regards retardation can be brought about by the combined action of moderate concentrations of carbon dioxide and of low temperature.

The remaining part of the experimental work may be divided into three sections, dealing with the combined action of carbon dioxide and low temperature upon (a) germination of fungal spores, (b) rate of growth of fungal colonies on artificial media, (c) rate of growth of specific fungi on certain fruits.

A. Rate of Germination of Fungal Spores at different Temperatures and in different Concentrations of Carbon Dioxide.

Experiments were carried out with spores of *Botrytis cinerea*, *B. parasitica*, *Penicillium glaucum*, and *Fusarium* sp. Sowings of spores were made on the following media:¹ potato agar, apple gelatine, prune gelatine, and Richard's solution with agar. These media were liquefied by heat and just before solidification were mixed with a few drops of a dense spore suspension, and then drops of the medium spread out on glass slides. The latter were then set up in moist containers and germination carried out in air and in air containing 10 per cent. and 20 per cent. CO_2 . One series was run at 15° and another at 5°. At intervals the average length of the germ-tubes was determined by micrometer counts. The carbon dioxide atmospheres

¹ The nutrient media were prepared in the usual way. Agar media had 1.5 % agar; gelatine media had 15 % gelatine. In the case of Richard's solution with agar, the nutrient and the agar require to be heated separately and mixed when nearly cool. The various plant decoctions were prepared according to the conventional standards suggested by Duggar (*Fungous Diseases of Plants*, p. 24, 1909).

were analysed at each measurement, and in no case was there found a deviation of more than 0.5 per cent. from the concentration required.

The incubation period and the rate of germination were found to vary to some extent with the different media employed, but the results obtained were of the same type for each medium and for each fungus. The following table gives the figures obtained for *Penicillium* on the two media, potato agar and apple gelatine:

TABLE VI.

Average Length of Germ-tube (in micrometer divisions).

		At 15°.	12 hrs.	16 hrs.	20 hrs.	23 hrs.	36 hrs.
Potato Agar.	{ Air		0.0	0.36	2.40	4.72	weft
	{ 10 % CO ₂		0.0	0.0	0.32	1.12	12.2
	{ 20 % CO ₂		0.0	0.0	0.0	+	4.84
Apple Gelatine	{ Air		0.0	0.70	2.44	5.12	weft
	{ 10 % CO ₂		0.0	+	0.44	1.10	12.0
	{ 20 % CO ₂		0.0	0.0	+	0.26	5.68

		At 5°.	1 day.	2 days.	3 days.	4 days.	5 days.	6 days.	7 days.	8 days.	9 days.
Potato Agar	{ Air		0.0	0.0	0.62	2.96	7.44	—	—	—	—
	{ 10 % CO ₂		0.0	0.0	0.0	0.0	0.82	2.96	8.84	—	—
	{ 20 % CO ₂		0.0	0.0	0.0	0.0	0.0	0.0	0.22	1.46	3.84
Apple Gelatine	{ Air		0.0	0.0	0.72	2.52	7.52	—	—	—	—
	{ 10 % CO ₂		0.0	0.0	0.0	0.0	0.12	—	—	—	—
	{ 20 % CO ₂		0.0	0.0	0.0	0.0	0.0	0.0	0.58	2.14	4.68

The above figures illustrate clearly a feature of the germination of fungal spores—that from the moment germination has begun, growth proceeds at a constantly increasing rate (at any rate for some time). Thus if we plot the average germ-tube length as ordinate against the time as abscissa, the curve of growth is convex to the x-axis.

The general quantitative effect of the two factors, low temperature and concentration of carbon dioxide, separately and combined, is perhaps better seen by comparing the time necessary for the average germ-tube to reach a given length in the different cases. Choosing a length = 5, and dealing with the germination tests on potato agar in the preceding table, we arrive at the following results:

Average germ-tube = 5 is reached in:

At 15° in Air	c. 24 hrs.
„ 10 % CO ₂	24–36 hrs.
„ 20 % CO ₂	36 hrs.
At 5° in Air	c. 4½ days
„ 10 % CO ₂	c. 6½ days
„ 20 % CO ₂	9 (+) days

These figures illustrate the magnitude of the effect produced. Even in the case of this very resistant fungus and in abundant nutrient, the effect of a 10° drop from ordinary temperature, combined with 10–20 per cent. CO₂, slows down the rate of growth in the early phases 6–9 times.

This method of measurement was, however, somewhat laborious and ill suited to the needs of the problem. Apart from the practical difficulty of carrying out the measurements sufficiently rapidly so as not to interfere too seriously with the various conditions of the experiment, there is the fundamental objection that the method of counting is severely restricted to the early phases of germination. When the average germ-tube reached a length of about ten divisions on the scale used, the method was no longer available except at the expense of excessive labour. It was therefore discarded in favour of the following one, which was much simpler to carry out and which at the same time gave results of much wider applicability and probably of more interest from the practical point of view.

B. Growth of Fungal Colonies at different Temperatures and in different Concentrations of Carbon Dioxide.

In these experiments plates of sterile medium, solidified with agar or gelatine, were 'poured' and inoculated at the centre with the fungus to be examined. In the case of *Sphaeropsis malorum*, the inocula consisted of mycelium only, as this fungus was found to sporulate very sparingly. For all the other fungi spore inoculations were in general used. It may be remarked here that, apart from a certain small difference in the amount of growth during the early stages, spore inocula and mycelial inocula gave rise to colonies of very similar appearance and rate of growth. The diameter of the colonies was measured directly by means of a small metric scale, and each measurement was made to the nearest half-millimetre.

As this method is extensively used in a paper dealing with the growth of fungal colonies under various conditions which will shortly appear, a detailed criticism of it will not be attempted here, but will be reserved till that occasion. The procedure is as follows: The plates, which contain a somewhat deep layer of medium (on the average, 1 cm.), are inoculated at the centre, care being taken to make the original inoculations as local as possible. They are then placed, face downwards and with lids off, in moist containers, four being placed in each of the latter. The Petri dishes are piled in column and separated from each other by means of wooden rods in such a way as to allow free access of the air of the container to the surface of each. For each temperature examined a comparison was made of the rate of growth in air, in air containing 10 per cent. CO_2 , and in air containing 20 per cent. CO_2 . The contents of each container were analysed for its carbon dioxide content at the time of measurement, this being especially necessary in the present series of experiments as the CO_2 content of each vessel tended to increase on account of the respiration of the fungal colonies within. This effect was negligible in the initial stages of each experiment, but became more pronounced as time went on, especially at the higher temperatures. It was desirable not to make the measurements

of growth too frequently, as each measurement involved a certain amount of disturbance of the experimental conditions intended. On the other hand, if the interval between successive measurements was too long, the accumulation of carbon dioxide in the containers tended to upset the experimental conditions aimed at. A compromise had therefore to be effected. From numerous analyses a fairly good idea of the rate at which carbon dioxide was formed in the containers was obtained, and from that it was possible to arrange to make each set of measurements at a time when the CO_2 content of each vessel did not exceed that originally intended by more than a certain percentage; in practice a limit of 2 per cent. was imposed. In the early stages of each experiment the deviation from the theoretical value at each time of measurement was much less than this limit, but towards the end of the experiment this limiting deviation was frequently reached. Thus in referring to growth in air, in 10 per cent. and in 20 per cent. CO_2 , what is really meant is growth in atmospheres containing 0.2 per cent., 10–12 per cent., 20–22 per cent. CO_2 respectively. The effect on growth produced by 2 per cent. CO_2 is small, and in any case what effect there is would only tend to make the results a little less pronounced than otherwise, seeing that the increase in CO_2 content due to respiration was always somewhat more rapid the lower the original CO_2 concentration in the container.

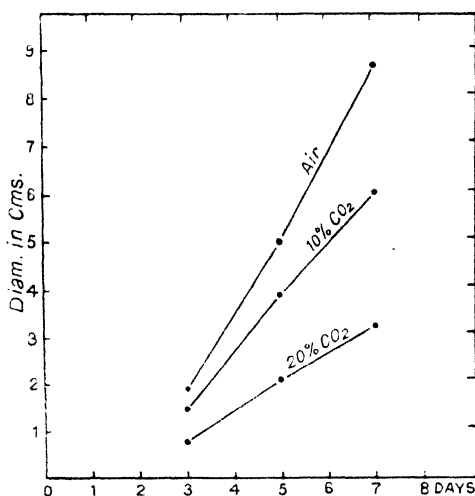


FIG. 1. Growth of *Botrytis cinerea* on apple gelatine at 15° .

All the fungi listed earlier in the paper were examined and the usual variety of media—potato agar, potato-, apple-, plum-, prune-gelatine, &c.—were employed. A series of experiments was run comparing the rates of growth in air, 10 per cent. and 20 per cent. CO_2 at 15° , 20° , and 25° ; a further series at 10° , 15° , and 20° ; and finally a more thorough comparison made of the rates of growth in the various atmospheres at 5° and 15° . A comparison was also made of the rate of growth of *Botrytis cinerea*, *Monilia cinerea*, *Alternaria grossulariae*, and *Fusarium* sp. in the various atmospheres at ordinary laboratory temperature (15 – 18°) with that at the ordinary cold storage temperature of 3° .

The general nature of the result will be seen by a study of Figs. 1 and 2, which represent the growth in diameter of colonies of *Botrytis*

cinerea at 15° and 5° on apple gelatine in air, 10 per cent. and 20 per cent. CO₂. All the cultures compared were started on the same day on the same medium, as was the invariable practice throughout this work. As the curves of growth at the two temperatures are drawn to different scale, the relative amount of retardation produced by the CO₂ treatment is not obvious at a glance, but an idea of its magnitude may be obtained by comparing the amounts of growth in the CO₂ cultures at times when the air cultures in the two cases have reached the same size. Thus if we read

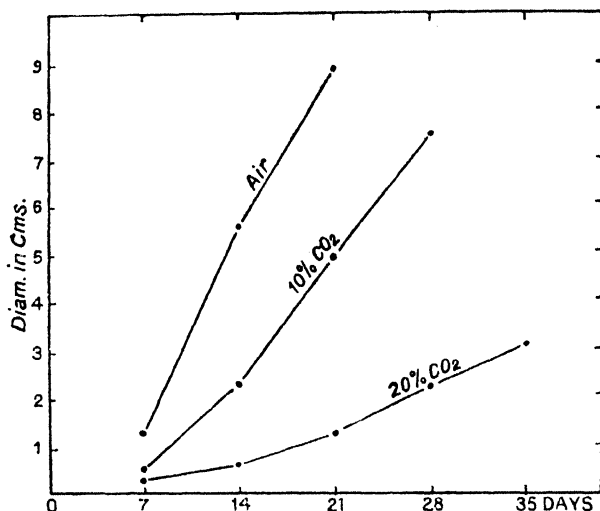


FIG. 2. Growth of *Botrytis cinerea* on apple gelatine at 5°.

off from the graphs the amount of growth in 10 per cent. and 20 per cent. CO₂ corresponding to a diameter of 8 cm. in the air cultures we get the following figures:

	Air.	10% CO ₂ .	20% CO ₂ .
At 15°	8.0 (100)	5.5 (69)	3.0 (37.5)
At 5°	8.0 (100)	4.3 (54)	1.1 (14)

The figures in brackets represent the relative amount of growth, that in air being taken as 100 in each case.

It will be observed that at the times when the air cultures at the two temperatures have reached the same diameter the corresponding amount of growth in the CO₂ atmospheres is considerably less at 5° than at 15°. This result may be expressed generally in the following terms: *The relative retarding effect of a given concentration of carbon dioxide is greater at a low than at a higher temperature.*

Strictly speaking, a correction should be made in the above curves for the diameter of the original inoculum, which in the case of *Botrytis* was

about 0.2 cm. The effect of making this small correction is to increase the relative retarding effect (as measured by dividing the growth in CO_2 by that in air), and more particularly is this the case for growth at the lower temperature.

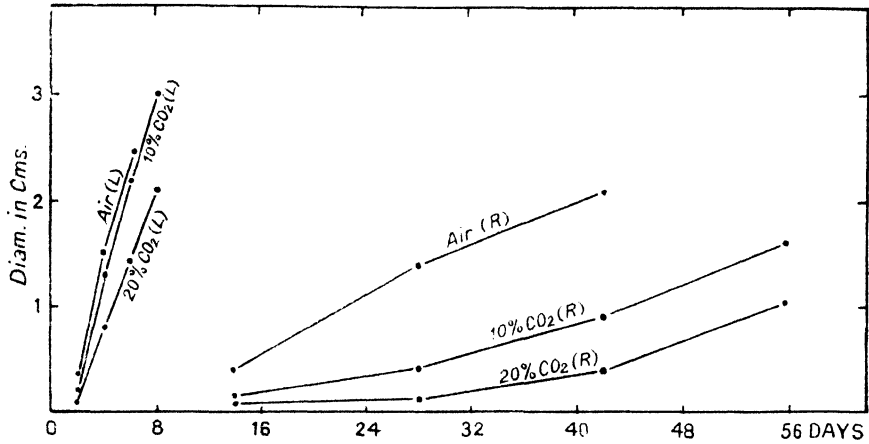


FIG. 3. Growth of *Botrytis cinerea* on potato agar at laboratory temperature (L) and in refrigerator (R).

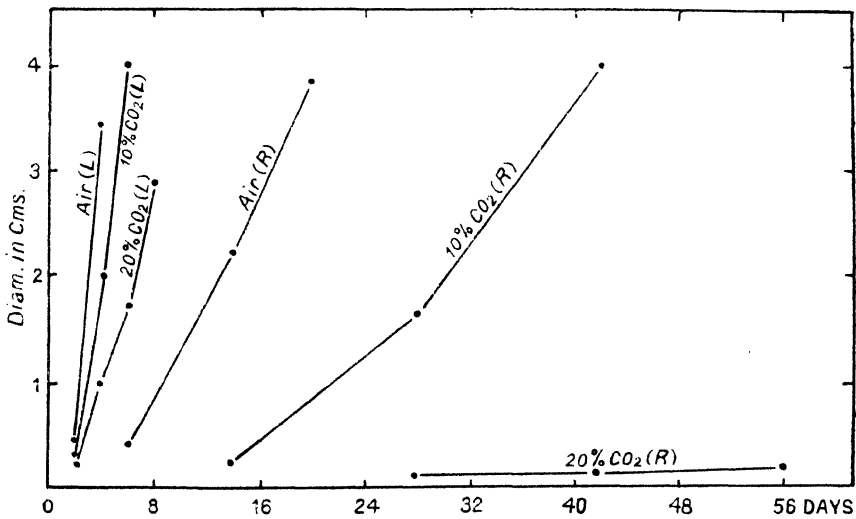


FIG. 4. Growth of *Fusarium* sp. on potato agar at laboratory temperature (L) and in refrigerator (R).

The effect under consideration becomes still more pronounced on comparing growth at 15° with that at temperatures lower than 5° , e.g. at 3° as in Figs. 3 and 4, which refer to experiments with *Botrytis cinerea* and *Fusarium* sp. on potato agar at laboratory temperature (15 – 18°)

and in a refrigerator maintained at 3°. On examining these curves along the lines indicated above we have the following comparison:

For *Botrytis*:

	Air.	10% CO ₂ .	20% CO ₂ .
At 15°	3.4 (100)	2.0 (59)	1.0 (30)
At 3°	3.4 (100)	0.7 (21)	0.0 (0)

For *Fusarium*:

At 15°	2.0 (100)	1.8 (90)	1.15 (57.5)
At 3°	2.0 (100)	0.82 (41)	0.36 (18)

Here in fact it is seen that in the case of *Botrytis* the low temperature combined with the depressing effect of 20 per cent. CO₂ is effective in almost stopping growth over a period of 56 days.

Similar curves were obtained for the growth of *Alternaria* and *Monilia* under the same set of conditions.

Considerable attention has been paid throughout this work to the study of the ratio

(growth in a given percentage of CO₂) : (growth in air).

The results arrived at may be stated as follows:

1. In the case of any particular fungus and for a given temperature this ratio tends to increase as time goes on; that is, the amount of retardation is greatest in the earliest phases of growth. For some fungi the ratio tends to reach a steady value, as is shown in the following table, which deals with the growth of *Alternaria grossulariac*.

TABLE VII.

Alternaria grossulariac. Growth on Potato Agar.

At 20°.	2 days.	3 days.	5 days.	7 days.	8 days.
Air	1.21 (100)	2.10 (100)	3.93 (100)	6.05 (100)	6.85 (100)
10% CO ₂	1.00 (83)	1.83 (88)	3.65 (93)	5.18 (86)	6.15 (90)
20% CO ₂	0.70 (58)	1.38 (66)	2.58 (66)	3.78 (62)	4.42 (64)
At 5°.	3 days.	5 days.	7 days.	10 days.	16 days.
Air	0.31 (100)	1.10 (100)	1.87 (100)	3.14 (100)	5.20 (100)
10% CO ₂	0.09 (29)	0.48 (44)	0.96 (51)	1.63 (52)	3.10 (58)
20% CO ₂	0.0 (0)	0.10 (9)	0.34 (18)	0.73 (23)	1.45 (27)

At both temperatures there is a tendency for the ratio of growth in CO₂ to that in air to increase. That the ratio is smallest at the beginning is well shown in the experiment at 5°, and is obviously related to the fact that in the early stages there is a period during which the air cultures show fairly considerable growth while the CO₂ ones have not as yet germinated. In such a case the ratio of growth in CO₂ to that in air is zero, but it begins to increase the moment the CO₂ cultures spread beyond the limits of the original inoculum. The same effect takes place at the higher temperature, but in the present experiment the first measurement was made too late to show the rapid rise of the ratio in the early stages. At both temperatures the ratio tends to reach a steady value. This has been

reached at the higher temperature by about the fifth day; at the lower temperature it has not been quite reached by the sixteenth day, though inspection of the table shows that the daily increase in the ratio is getting less and less. At the higher temperature the ratios of growth* in 20 per cent. CO_2 : growth in 10 per cent. CO_2 : growth in air, tend to reach the limiting values 65: 85-90: 100, whereas the corresponding ratios for growth at 5° are in the neighbourhood of 30: 60: 100.

These tables again illustrate the effect of a lowering of temperature on the retardation of growth produced by a given concentration of carbon dioxide.

In the case of some fungi, and especially when they are sown on certain media, the ratio of growth in CO_2 to that in air goes on increasing with time without showing any tendency to reach a limiting value, at least within the limits of size to which the colonies have been studied. In such cases as these the growth of colonies in the CO_2 concentrations finally exceeds that in ordinary air, especially at higher temperatures. The following table dealing with the growth of *Fusarium* will illustrate this point:

TABLE VIII.

Fusarium. Growth on Potato Agar.

At 15° .	3 days.	5 days.	7 days.	8 days.
Air	1.23 (100)	2.23 (100)	2.98 (100)	3.3 (100)
10% CO_2	1.1 (85)	2.4 (108)	3.5 (118)	4.04 (121)
20% CO_2	0.95 (73)	2.1 (94)	3.3 (111)	3.8 (115)
At 5° .	7 days.	14 days.	21 days.	35 days.
Air	0.4 (100)	1.6 (100)	2.2 (100)	3.85 (100)
10% CO_2	0.1 (25)	0.8 (50)	1.3 (59)	2.5 (65)
20% CO_2	0.0 (0)	0.05 (3)	0.18 (8)	0.7 (18)

The present case brings out best of all the effect of temperature on the retarding action of carbon dioxide. Corresponding to a colony diameter of 3.85 at the lower temperature, there is very considerable retardation in the CO_2 atmospheres, whereas at the higher temperature the colonies in 10 per cent. and 20 per cent. CO_2 are distinctly ahead of the colonies in air for some time before the latter reach that value. It is not proposed to discuss this effect here, as it will be dealt with more fully in a later paper, but it may be stated that it is related to the marked 'staling' which takes place in the air cultures. The main feature of 'staling' in the present case is the development of alkalinity by the fungus, and this is partly counteracted by the action of carbon dioxide. Hence while the air cultures at 15° become progressively slower in growth, the CO_2 cultures keep up a steady rate of growth for a longer time, with the result that the initial retardation produced by carbon dioxide is sooner or later converted into an acceleration.

As will be shown more fully in a subsequent paper, the particular

effect just described is more markedly shown by cultures on potato agar, though it is also seen on other media. Also it is met with in the case of other fungi which show marked staling on potato agar. Such fungi are, in addition to *Fusarium*, *Sphaeropsis malorum* and more markedly *Colletotrichum Lindenmuthianum*. The effect is not seen with fungi such as *Alternaria grossulariae*, *Botrytis cinerea*, &c., which show no such marked development of alkaline reaction on potato medium.

2. On comparing different fungi with each other in respect of the amount of retardation produced by a given concentration of carbon dioxide, it was seen that some are more susceptible than others. One could thus arrange the various fungi in a series of diminishing susceptibility, and this series was roughly the same as that given on p. 283, which is based on the concentration of carbon dioxide necessary to inhibit spore germination. In constructing such a table on this principle, one is limited to a consideration of the early phases of growth, as the staling effect by and by comes in as an important disturbing factor. However, it is quite possible to show that at ordinary temperatures a given concentration of carbon dioxide exerts a greater retarding effect on *Botrytis cinerea* than on *Sphaeropsis malorum*, at all stages of growth on all the media tested. When, on the other hand, the experiment is repeated at 5° the reverse is found to be the case. Whereas *Botrytis* colonies grow at 5° in 20 per cent. CO₂, *Sphaeropsis* colonies under these conditions are practically inhibited. The explanation of this is probably to be found in the different temperature minima of the two fungi. The minimum for *Botrytis* is very near 0°, and that for *Sphaeropsis* was shown to lie between 3° and 5°. The greater effectiveness of 20 per cent. CO₂ in the case of the latter fungus is probably correlated with the fact that the experimental temperature is approaching very close to the minimum for the fungus. It is reasonable to suppose that within a few degrees of the minimum temperature a moderate concentration of carbon dioxide is effective in producing not merely retardation but total inhibition of growth. Thus the region of total inhibition by 20 per cent. CO₂ is already reached at 5° in the case of *Sphaeropsis*, whereas a lower temperature has to be sought in the case of *Botrytis*.

3. The amount of retardation of growth at a low temperature produced by a given concentration of carbon dioxide is different according as the colonies have been grown throughout at the low temperature or were started at a higher temperature. The quantitative effect produced here appears to depend considerably on the particular medium used. The following table refers to *Botrytis cinerea* inoculations on apple gelatine :

(a) *B. cinerea* grown throughout at 5°.

Diameter of growth after 23 days :

Air.	10% CO ₂ .	20% CO ₂ .
7.6 (100)	3.35 (44)	0.85 (11)

(b) Cultures grown for two days in air at 20°, then transferred to 5°, some in air, some in 10 per cent. and 20 per cent. CO₂.

Diameter of growth in 14 days after transference:

Air.	10% CO ₂ .	20% CO ₂ .
6.75 (100)	5.2 (77)	2.65 (39)

In the latter case the retarding action of carbon dioxide is less than in the former, and this is obviously due to the fact that in the latter case, by germinating the spores in air at a high temperature, they were taken past the stage at which the retarding effect of carbon dioxide on them is greatest.

A similar behaviour to that of *Botrytis* was shown by *Monilia* and *Fusarium* on apple gelatine.

With potato agar, very diverse results were obtained with different fungi. *Botrytis cinerea* behaved in the same way on this medium as on apple gelatine. On the other hand, *Penicillium* and *Sphaeropsis*, after being grown for two days at 20° and then transferred to 5°, had their growth in air stopped entirely for a long time at the latter temperature, whereas they continued to grow slowly in 10 per cent. CO₂. This complicated behaviour is again without doubt associated with the fact that potato agar is a medium which lends itself with particular readiness to staling by development of alkalinity.

C. Rate of Growth of certain Fungi on Fruit at different Temperatures and in different Concentrations of Carbon Dioxide.

Experiments were carried out on the rate of attack of apple by *Botrytis cinerea* and *Monilia cinerea*, and of orange by *Penicillium glaucum*. The fungi were introduced through artificial punctures in the skin of the fruit. Parallel series were run at 15° and 5°. Throughout the experiment it was necessary to open the containers every second day at the higher temperature and every four days at the lower temperature, and the atmospheric composition was made up afresh, this by reason of the respiration of the fruit. At the end of the experiment the amount of rotting in the case of apple was determined by weighing before and after removal of the rotted portion, and in the case of orange by counting the percentage of loculi affected by the fungus. The latter method did not appear to be very satisfactory. The following table gives the percentage of rotted tissue:

TABLE IX.

	Air.	10% CO ₂ .	20% CO ₂ .
At 15°. After 10 days.			
<i>Botrytis cinerea</i> . .	14.6	1.8	0.4
<i>Monilia cinerea</i> . .	63.5	23.3	13.7
<i>Penicillium glaucum</i>	53	52	45
At 5°. After 28 days.			
<i>B. cinerea</i>	13.3	1.0	0.05
<i>M. cinerea</i>	45.9	11.4	1.0
<i>P. glaucum</i> (35 days)	50	—	26

A comparison of the figures obtained at the two temperatures of the experiment again shows that the relative retarding effect of a given concentration of carbon dioxide is accentuated by lowering the temperature. The comparatively high degree of retardation produced by carbon dioxide on the rate of *Botrytis* attack as compared with that of *Monilia* would seem to be correlated with the less vigorous parasitic action of the former on apple.

DISCUSSION.

As the main part of the foregoing experimental work deals with the rate of increase in *diameter* of fungal colonies, a brief discussion of the method of measurement adopted is necessary here, though a fuller criticism is reserved for a future occasion.

For the present investigation (as for many others of a physiological nature) the really important quantity to be determined in connexion with fungal growth is the amount of work done, i.e. the amount of chemical transformation carried out. This quantity, which would be very difficult to determine directly, is in all probability closely related to the amount of growth made by the fungus. There is thus a rough proportionality between the amount of chemical transformation effected by the fungus and the amount of fungal mycelium. The question now arises as to the relation between the amount of mycelium in a fungal colony and the diameter of the latter. As between different media, or even different concentrations of the same medium, there is no relation between the amount of mycelial growth and the diameter. On some media a given fungus may produce a dense web with slowly advancing margin, and on others a thin colony with rapidly increasing diameter. In such a case measurement of the diameter is of no value in determining the amount of growth made by the fungus in the different cases.

When, however, comparisons are made between growths under different conditions on the same medium, definite conclusions as to the amount of mycelium can be drawn from a measurement of the diameter of the colony. With any particular medium, the type of growth produced by the same fungus is very similar under the different conditions of experiment, whether in air or in concentrations of carbon dioxide, or whether at laboratory temperature or in cold store. With fungal colonies growing on the same medium, the diameter of the colony is a measure of the amount of growth in the sense that a greater diameter represents a greater amount of mycelium than does a smaller diameter, and conversely.

The exact relation between diameter of colony and amount of mycelium is not certain, and almost certainly varies with the age of the colony. If the colony grew spherically on the medium (or on the fruit) the amount of mycelium would be more or less proportional to the cube of the diameter, but, as is noted by Brooks and Cooley (4), the colony always spreads more

rapidly tangentially than in a direction normal to the surface, and therefore the amount of mycelium will be proportional to the diameter raised to a power intermediate between two and three. Thus it will be seen that if the foregoing results are expressed in terms of 'amount of mycelium in the colony', the effects become more marked in each case, even if we only assume that the amount of mycelium is proportional to the square of the diameter.

The main conclusion arising from the experimental work detailed in this paper is that the retarding effect of a given concentration of carbon dioxide on germination and growth is greater at a low than at a high temperature. The question arises as to what causes this differential effect can be assigned. A similar effect was obtained by Kidd for the action of carbon dioxide on the germination of seeds, and the nature of the action briefly discussed. As is pointed out by Kidd, the important factor is probably the tension of carbon dioxide within the living protoplasm, and this is controlled to a large extent by the pressure of carbon dioxide in the surrounding atmosphere. For the case of a fungus growing within the tissue of a fruit such as an apple, the factors which influence the carbon dioxide content of the fungal hyphae (respiration of the fruit, respiration of the fungus, rate of gaseous diffusion, &c.) are so various and complicated that any discussion of them would be useless. However, the differential retarding effect under consideration was shown not only for the rate of attack of fruit, but also for the rate of growth of fungus colonies on artificial media, in which case the phenomenon is obviously simpler, and to which case the discussion may be confined.

The concentration of carbon dioxide within a fungal hypha growing in a given atmosphere of carbon dioxide depends upon:

1. The solubility of carbon dioxide in water.
2. The rate of respiration within the hypha and the rate of gaseous diffusion across the hyphal membrane.

The solubility of carbon dioxide in water at 5° is to that at 15° in the ratio 1.4 : 1 (according to Landolt-Börnstein). If the concentrations of carbon dioxide in the growing hyphae at the two temperatures stand to each other in this ratio, then it is difficult to see that the retarding effects obtained can be completely explained on this ground. On reference to the tables given in the present paper it will be seen that the retarding effect of a given concentration of carbon dioxide at 5° is in many cases very much greater than that shown at 15° . Furthermore, the relative retardation shown varies from fungus to fungus on the same medium, and is strongly influenced by the amount of nutrient present and by the proximity of the temperature under consideration to the minimum temperature for the particular fungus. It is difficult, therefore, to see how these various effects can all be explained on the grounds of this one factor.

The effect of respiration would be to make the concentration of carbon dioxide within the hypha somewhat greater than that in the nutrient medium immediately surrounding the hypha. The actual difference in concentration would depend on the rate of production of carbon dioxide within the hypha and its rate of diffusion through the hyphal membrane, both of which are accelerated by increasing temperature. According to Kidd, one is probably not far wrong in assuming that the variations of the two factors with temperature neutralize each other, so that the carbon dioxide content of the growing hypha would be approximately the same at different temperatures. It does not appear, therefore, that the differential retarding effects described in this paper can be explained on the basis merely of differential carbon dioxide content of the hyphae under the different experimental conditions.

Summarizing the above argument, we may say that the greater retarding effect of a given concentration of carbon dioxide at a low than at a high temperature can be partly explained on the basis of the greater solubility of carbon dioxide in water at the low temperature, but over and above that other physiological factors come into play, the precise nature of which is at present unknown.

Whatever be the physiological explanation of the various results obtained, it is possible to put forward an hypothesis that brings all the experimental results into a coherent form. The more important factors influencing growth are as follows:

1. *Nutrient.* The rate of growth increases with the concentration of the nutrient up to certain limits.
2. *Temperature.* The rate of growth increases with rise of temperature up to a certain limit, and as growth is nil at a minimum temperature, the rate of growth is obviously some direct function of $(t - t_0)$, where t is the particular temperature and t_0 is the minimum temperature.
3. *The amount of growth already made.* The relation of growth to this factor is complex, but up to a certain point it is such that the rate of growth increases with the amount of growth already made.
4. *Density of spore suspension of the fungus.* This factor becomes prominent in the case of spore sowings in weak nutrient and acts in such a way that growth diminishes with increasing density of spore suspension.
5. *The percentage of carbon dioxide in the atmosphere.* As far as can be seen this is always a retarding factor in the case of fungal growth, except in cases where alkaline 'staling' takes place and accelerated growth is the result.

Considering the first four factors which constitute the environmental conditions of the fungus from the point of view of the carbon dioxide treatment, we may say that the energy of growth is proportional (in some ratio) to the concentration of nutrient, to the temperature, to the amount

of growth already made, and to the diluteness of spore suspension (all within limits). All the experimental results obtained will then fall under the following general rule, viz. *that the carbon dioxide retarding factor has greatest effect when the energy of growth is small*. This general statement will cover all the experimental results obtained for the retarding effect of carbon dioxide on growth at different temperatures, in different concentrations of nutrient, and at different stages of growth. It will also include certain results obtained by comparing the retarding effect of carbon dioxide on the growth of some fungi on living fruit with that on artificial medium. On referring to Table IX, it will be seen that at 15° a given concentration of carbon dioxide more effectively retards the parasitic attack of *Botrytis cinerea* on apple than is the case with attack by *Monilia cinerea*. On artificial media both fungi were seen to be about equally sensitive to the action of the fungus. Now *Botrytis* is a weaker parasite on apple than is *Monilia*, and though a large number of factors may play a part in producing this result, one can state generally that *living* apple tissue is a poorer nutrient for *Botrytis* than for *Monilia*. Thus the figures shown in Table IX can be brought into line with the general rule that the retarding effect of carbon dioxide is greater the poorer the nutritive value of the substrate for the particular fungus. Some of the results of Brooks and Cooley already cited will also be seen to be interpretable along the lines of the general rule here put forward.

The following practical considerations which arise from the foregoing work may now be briefly described:

1. Within the limits of carbon dioxide concentration admissible in practice, carbon dioxide is not so important a factor in reducing the amount of fungal growth as is temperature. At ordinary temperatures a concentration of carbon dioxide as great as 20 per cent. is not so effective in controlling fungal growth as is a drop of 10° without carbon dioxide.

2. The efficiency of the carbon dioxide treatment depends on the amount of nutrient available for the fungus. A concentration of carbon dioxide of 10-20 per cent. would give a good effect on spores devoid of nutrient and a much less effect on spores which had free access to nutrient, and less still when attack had already started. The nutritive conditions obtaining on the surface of stored fruit will obviously depend on a number of factors—the particular kind of fruit, the soundness of the fruit, &c. Where the fruit is injured in any way, nutrient will always be available to any fungal spores which happen to be present, but even under the best conditions of storage a certain amount of nutrient will always be available to fungal spores (at least in the case of apple), as it has been shown in unpublished work of the writer that certain volatile products of the apple have a very distinct accelerating effect on spore germination. The food available to the fungus is therefore never negligible. As regards the effect

of the carbon dioxide treatment on the germination of fungal spores on fruit in store, one would expect a result intermediate between that obtained for germination in water and germination in a good nutrient.

3. The efficiency of the carbon dioxide treatment is enhanced by lowering the temperature. Certain cases have been alluded to where a moderate concentration (10–20 per cent.) of carbon dioxide at ordinary temperature accelerates fungal growth, and though this result was only obtained for growth on certain artificial media, it is not at all certain that a similar effect might not arise if the storage of certain fruits or vegetables were attempted at ordinary temperatures in carbon dioxide. The present experiments thus indicate that, as far as concerns fungal attack in the store, the gas storage method is to be considered as an aid to the ordinary method of cold storage, and as in no way replacing the latter.

SUMMARY.

1. Within very wide limits, variation of oxygen pressure has little effect on the germination and growth of the ordinary fruit-rot organisms such as *Botrytis*, *Fusarium*, and *Alternaria*.

2. The germination and growth of these organisms is retarded by carbon dioxide. The retarding action of carbon dioxide on germination and growth is more marked the lower the temperature, the weaker the nutrient in which the fungal spores are sown, and, to a less degree, the greater the density at which the spores are sown.

3. The concentration of carbon dioxide which inhibits germination at ordinary temperature was determined for the commoner fruit-rot organisms both for germination in water and in nutrient.

4. Graphs and tables are given of the rates of growth of a number of fungi at various temperatures and in various concentrations of carbon dioxide.

5. The experimental results indicate that the gas storage method is most effectively used in combination with the ordinary cold storage method, and that it will give the best results when no attack of the fruit has begun previous to storage, and when conditions are such that a minimum of nutrient is available to fungal spores on the surface of the fruit.

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Studies in the Physiology of Parasitism. IX. The Effect on the Germination of Fungal Spores of Volatile Substances arising from Plant Tissues.

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IN the course of an investigation of the exosmosis of nutrient substances from plant tissues into the infection drop (1), the observation was made that spores of *Botrytis cinerea* germinate better when petals of *Rosa centifolia* are placed in the same Petri dish, but out of contact with the water drops in which the spores are sown, than when they are absent. This remained an isolated observation for some years. When the subject was again taken up it was found that the capacity to stimulate¹ and, more generally, to influence the germination of fungal spores in some way or other, by means of some volatile emanation could be demonstrated in the case of a large number of plants.

To the writer the main interest of these observations lies in the possible effect which volatile substances of plant origin may have in the establishment or non-establishment of fungal attack. It is felt, however, that it will be impossible to estimate their importance in questions of immunity and susceptibility until certain other nutritional problems have been dealt with. For this reason a more intensive study of these effects, and especially in relation to the chemical nature of the substances which produce the phenomena observed, has been postponed for the time being. Though the present account is of a somewhat preliminary nature, nevertheless enough work has been done to show that volatile substances of plant origin may play a

¹ The term 'stimulation' is here applied in the simple descriptive sense of increased germination; for the converse effect of reduced germination the word 'retardation' is generally used. It is fully recognized that effects of 'retardation' are also effects of 'stimulation', in particular of too strong stimulation in the usual physiological sense. Further, the use of the term 'stimulation' is not to be taken as prejudging the question as to whether the results are of a stimulatory as contrasted with a nutritional nature. The distinction between stimulus and nutrient is more easily drawn in theory than in practice, and in the case of the substances considered in the present paper—which most probably contain carbon and thus may enter directly into the carbon metabolism of the fungus—any satisfactory distinction between stimulus and nutrient is at present impossible.

considerable, and a hitherto scarcely suspected, part in influencing the germination of the spores of fungal parasites.

The most significant statement in the literature dealing with this subject is that of Neger (2), who observed that spores of *Bulgaria polymorpha* germinate better in hanging-drop cultures when pieces of oak, beech, &c., bark or wood, were placed in the bottom of the cell than when they were absent. Neger makes no statement as to the nature of the substances producing this effect.

As we are undoubtedly concerned here with volatile organic substances which produce stimulation of germination when present in very low concentration, reference may be made to the work of Duggar (3), who states that traces of ethyl alcohol or ether cause increased germination of the spores of *Aspergillus*. (Duggar further records that distilled water which has been kept for some time in paraffin-lined flasks produces better germination of the spores of *Aspergillus flavus* than similar distilled water which has not been in contact with paraffin. Similarly the presence of vaseline in the hanging-drop cultures was found to improve the germination of *Aspergillus flavus* spores by 10 to 20 per cent.)

As a striking illustration of the influence of small traces of a volatile organic substance, we may cite the work of Beijerinck and van Delden (4), who describe a bacterium, called by them *Bacillus oligocarbophilus*, which did not grow in a nutrient containing a source of carbon food, but made appreciable growth in the absence of the latter from the cultural solution. These workers showed that the source of carbon in the case of this bacterium was some volatile carbonaceous substance present in the atmosphere, but which was not carbon dioxide. As to the nature of this substance, they were not able to make any statement beyond the fact that it was more abundant in laboratory than in greenhouse air.

The usual method of experiment in the present work was to lay the plant organs in question in large Petri dishes side by side with clean slides on which drops of an aqueous ^{water}suspension of spores were placed. In a limited number of experiments, as will be mentioned later on, the plant structures were placed in the bottom of large containers and the slides placed in a rack above. As in general the effects were more marked when the leaves, &c., were first bruised, the rule was made to put the leaves into the Petri dishes first, and then after a time the slides, so as to avoid any risk of droplets of plant juice falling on the slides. Also the bruising operation was always done at some distance from the Petri dishes, in most cases in another room. Petals of *Rosa centifolia* were always washed first of all to remove any adherent pollen.

As regards the cleaning of glassware, preparation of spore suspensions, &c., the usual methods were adopted. Ordinary distilled water was used throughout. In the earlier stages of the work the Petri dishes were washed

after each experiment and allowed to dry out completely in the open air of the laboratory. This was to ensure that all traces of volatile substances from the preceding experiments had become dissipated before the dishes were used again. Latterly the method was adopted of strongly heating the dishes in a dry-air oven.

In the course of the investigation it was found that the moist filter- or blotting-paper, which it is usual in experiments of this kind to insert in the lids of Petri dishes for the purpose of preserving a moist atmosphere, produces a distinct effect on the germination of spores in the Petri dish. This effect will be described in the latter portion of this paper, but here it may be stated that filter- or blotting-paper which has lain moist in the lid of a Petri dish for some days gives off a volatile substance which reduces the amount of fungal germination taking place in pure water or in very dilute nutrient. Throughout the greater portion of this work parallel series were run, the one of Petri dishes set up with moist filter- or blotting-paper in the usual way, the other in which no such paper was present. In the latter case drying up of the drops was prevented by liberal moistening of the inner surface of the Petri dish.

It may be added that this paper effect proved to be of value from the experimental point of view, as it afforded a means of greatly reducing or actually suppressing the amount of germination which takes place in pure water in the case of some fungal spores. The stimulating effect arising from plant tissues becomes then more striking, as will appear from the tables given below.

In each experiment at least two, and usually four, Petri dishes were employed for each particular treatment. In each Petri dish two to four slides, each with two drops of the same spore suspension, were placed. Thus in each case abundant provision was made for accidental variations. The amount of germination was determined by measuring the length of germ-tube of a large number of spores (usually twenty-five from each drop) chosen at random from the central region of the drop, and dividing the total length of the germ-tubes by the number of spores counted. The restriction as to measuring germ-tubes from the central region of the drop is rendered necessary from the fact that under conditions of feeble nutrition germination is always somewhat better at the margins than in the centre of the drop. This point is also noted by Duggar in the paper referred to.

In practice it was found impossible to measure quantitatively the germination in all the drops which it was customary to set up. The number of Petri dishes used in any one experiment usually ranged from twenty to fifty, each containing four to eight drops, so that it was manifestly impossible to measure satisfactorily the amount of germination in each particular one. The large number of tests enabled the experimenter to arrive at a general impression of the result, and then as many measurements

were made as it was judged would give an adequate representation of this impression. In many cases the differences were so pronounced that a limited number of measurements served for this purpose. Where the differences were less marked, more measurements were taken. In some cases, where differences appeared over even a large number of measurements, and where examination of all the drops gave no definite impression of difference, the results of measurement were ignored. The conclusion in such cases was that differences, if they did exist, were not large in amount, and therefore, in exploratory work such as the present is, the fuller examination of such effects should be postponed until such time as it appeared that the subject demanded it. The claims made in the present paper are on a very conservative basis, and are confined to the major and obvious results met with in the course of the work.

Most of the observations were made with spores of *Botrytis cinerea*, which proved in all respects to be the most convenient fungus for this work. Tests were also made with spores of *Botrytis parasitica* (*Allii*, the form occurring on onion), *Monilia fructigena*, *Penicillium glaucum*, *Fusarium* sp., and *Colletotrichum Lindemuthianum*.

The list of plants tested was as follows: petals of *Rosa centifolia*, leaves of *Ruta macrophylla*, *Santolina Chamaecyparissus*, *Eucalyptus globulus*, *Pelargonium zonale*, *Choisya ternata*, *Chrysanthemum* (garden vars.), *Lavandula spicata*, *Foeniculum vulgare*, *Fuchsia* (garden var.), *Vicia Faba*, *Melittis melissophyllum*, *Amomum cardamomum*, *Andropogon Schoenanthus*, *Allium Schoenoprasum*, *Agapanthus umbellatus*; bulb scales of onion; fruits and leaves of apple; rind of orange fruit; and tubers of potato.

The following table is typical of the result obtained when a few (one or two) bruised leaves are placed in Petri dishes side by side with slides on which lie drops of a suspension in water of spores of *Botrytis cinerea*. Moist blotting-paper was present in the Petri dish-lids in all cases. Spores of two different ages were used, the older giving feeble germination in water. In the case of the controls the figures are the average of 100 counts; the others are based on 50 counts. The measurements of germination were taken two days after the start of the experiment.

TABLE I.

	Old spores.	Young spores.
Control	0.07	2.03
Amomum	0.94	3.48
Eucalyptus	0.52	2.60
Santolina	0.60	3.44
Ruta	1.74	3.68
Allium	0.00	0.00
Choisya	2.16	5.08
Lavender	0.32	similar to control
Agapanthus	0.96	3.08
Apple (leaves)	2.16	5.00

Tests of unbruised leaves were run simultaneously, but only in the following cases was an increased germination noted :

	<i>Old spores.</i>
Control	0.07
Ruta	0.25
Choisya	0.35
Apple	0.22

A large number of experiments have been carried out along the lines of the one above described, and, while the results vary in degree in different experiments, the general result is convincing, viz. that certain volatile substances given off from plant tissues—especially when the latter are bruised—have a distinct effect on the germination of fungal spores. In the majority of cases the effect was found to be one of increased germination, but in some instances a distinct retardation, amounting in some cases to a complete inhibition, was produced. The most striking illustration of the latter so far observed is that of the onion. Tests have been made of leaves of *Allium Schoenoprasum* and of bulb scales of the ordinary onion, and in all cases it has been found that the addition of a small piece of onion tissue has a strongly repressing effect on the germination of *Botrytis* spores. This result is in agreement with the statements of Walker (5) on this point.

It is impossible at present to assert that one particular plant produces greater stimulation than some other one, as no means has been devised for regulating or measuring the intensity of the stimulating factor. Quite apart from questions of the varying amount of tissue and the varying amount of cell-bruising in different cases, there is also the different volatility of the various volatile compounds, their various solubilities in water, &c. In practice it has been found that sometimes a particular plant gives the most striking result, in other experiments some other plant. But while no exact rule can be laid down in this respect, one can say broadly that some plants give good stimulation, others less so. Good material for showing stimulation are bruised leaves of *Choisya*, *Ruta*, and apple, though equally good results have been at times obtained with leaves of *Eucalyptus*, *Anomum*, and some others. It will be noticed that most of the plants tested are of an aromatic nature. The effect, however, is not confined to such oily plants, for distinct stimulation of growth has been obtained with bruised leaves of broad bean. In this case it was found that a considerable amount of tissue was required. On the other hand, only small stimulation has yet been seen in the case of lavender leaves.

Table I brings out an interesting experimental point, viz. that the stimulative effect is more readily discernible when the spores are of feeble germinative capacity. The ideal condition is when the spores in the control experiment remain ungerminated, in which case any stimulative effect is

obvious at a glance. With young vigorous spores which may give an average germ-tube length of two to four divisions of the micrometer scale in the control tests, the result is not so obvious. On the other hand, spores of vigorous germinative capacity are desirable where a retarding effect is concerned. In many experiments, therefore, the practice has been to use both vigorous and feeble spores, so that suitable conditions were present for detecting both retarding and stimulating effects.

The vigorous type of spore is obtained from 1-2 week old cultures on potato agar: the feeble spores from cultures of 4-8 weeks' age. In very old cultures still feeble germination is obtained, but the individual variations among the spores of a culture become more and more pronounced, not only when the spores are sown in water, but also when they are germinated in a good nutrient. This lack of uniformity places a limit beyond which the spores cannot be profitably used.

A method that proved useful for ensuring small germination in the control tests was to make use of the retardation due to blotting-paper already mentioned; this point will be illustrated later.

Apart from the effect of stimulation which results in an increased average germ-tube length, a distinct formative effect has been noted in many cases. This takes the form of increased stoutness of the germ-tube. In such cases it is not possible to give an adequate quantitative expression of the result. The stimulated and the control spores may on measurement give very much the same average germ-tube length, though the appearances presented in the two cases may be very different. In some cases, as will be noted subsequently, it is not possible to describe the effect either as a simple stimulation or a retardation, since the control germ-tubes take the form of long thin hyphae, whereas those formed in the presence of the plant tissue are shorter but much stouter.

Experiments have been carried out showing that the volatile stimulants are sufficient to increase markedly the parasitic powers of *Botrytis* spores. An experiment along these lines gave the results shown in Table II.

The experiment was set up in the general way already described, with the difference that several leaves of broad bean were put in, each leaf having on it a number of drops of the same spore suspension as was used for studying the amount of germination on the glass slides. The figures in the last column are in the form of fractions, the denominator of which gives the number of inoculations, the numerator the number of infections which had taken on the second day from sowing, at which time all the readings in the table were taken. It may be remarked that the increased parasitic effect was greater than the figures would indicate, as the greater percentage of infections in presence of the plant tissue was accompanied, on the average, by a much more advanced attack.

The effect produced by bruised bean leaves was only got by adding an

amount of bruised leaf tissue much greater than was found to be sufficient in the case of the other plants mentioned.

TABLE II.

<i>Leaves tested.</i>	<i>Av. germ-tube.</i>	<i>Attack of Bean leaves.</i>
Control	0.18	6/28
Choisya	3.69	14/17
Ruta	4.42	10/11
Amomum	0.95	17/24
Foeniculum	0.97	11/20
Pelargonium	1.28	14/15
Apple	2.48	16/20
Chrysanthemum	1.20	7/14
Broad bean	2.34	13/14

Special attention was given in this work to volatile substances given off by the tissue of apple fruit and potato tuber. The former of these gives distinct stimulation, the latter retardation of the germination of *Botrytis* spores.

As regards the cause of these effects, it was thought conceivable that they might be due to the action of the carbon dioxide of respiration. It was known that carbon dioxide represses germination if present in sufficient concentration (6). Also the possibility had to be considered that a slight concentration of carbon dioxide might counteract any slight alkalinity arising from the glass of the slides, it being known that a very slight degree of alkalinity had a pronounced inhibiting effect on the germination of *Botrytis* spores. The following experiment with apple slices shows that carbon dioxide is not the factor concerned.

Four thoroughly washed glass containers of three litres capacity were used. The various liquids employed (50 c.c. of each) were placed in glass vessels in the bottom; the apple slices, approximately the same weight in each, also in the bottom, and the slides (four in each) with the spore drops laid on a rack above. After two days the CO₂ content of each container was analysed and the amount of germination determined in each drop. The following were the results obtained:

TABLE III.

<i>Container with:</i>	<i>CO₂ content.</i>	<i>Average of 4 counts of 25 spores each.</i>
50 c.c. H ₂ O: no apple	0.0 %	0.34 (0.14 to 0.44)
" : apple slices present	3.3 %	1.80 (1.38 to 2.12)
50 c.c. dil. NaOH: " "	0.0 %	2.32 (2.18 to 2.44)
50 c.c. dil. H ₂ SO ₄ : " "	3.1 %	2.25 (1.72 to 2.76)

The amounts of germination in the last three are all of the same order of magnitude. The figures in brackets give the limits of variation in the different counts. The germination in the absence of apple is distinctly least

in all cases. This experiment proves conclusively that the carbon dioxide evolved by the apples is not the factor affecting the germination.

An experiment in which the germination in air was tested against that in 3 per cent. CO₂ artificially made up gave the following result:

In air	Germination = 0.44
In 3 per cent. CO ₂	„ = 0.30

This further confirms the foregoing result.

A similar conclusion was reached for the retarding effect shown by potato tissue.

The stimulating effect of apple can be obtained with slices, with bruised, or with unbruised whole apples. As far as could be seen, the effect of the last mentioned was just as great as that of any of the others. The conclusion is thus reached that healthy unbruised apples can markedly stimulate spore germination in their vicinity.

In some tests an attempt was made to vary the amount of stimulation by using 1, 2, 5, &c., slices of apple, and as the final member of the series a considerable amount of pounded apple tissue. A distinct optimum effect was in some cases observed, that is, reduced germination was again met with in the last case. This optimum effect will be alluded to later in dealing with stimulation by ethyl acetate. In the present case it was not certain that fermentation in the pounded-up tissue may not have played a part in the result.

The following experiment, carried out in large containers, may be cited as confirming a statement already made, viz. that the effects described are more clearly discernible with somewhat feebly germinating spores than with vigorous ones.

TABLE IV.

<i>Apple.</i>	<i>Spores.</i>	<i>Germination.</i>
Absent	Old	0.61
„	Young	1.50
Present	Old	2.43
„	Young	3.28

The ratio of stimulated to control spores is 4.0 for the old spores and 2.2 for young spores. The effect of stimulation is thus more striking in the former case.

The following somewhat detailed experiment may be quoted as illustrating among other things the retarding action arising from blotting-paper. The plants tested were: bruised leaves of *Eucalyptus*, slices of apple fruit and of potato tuber, and strips of rind of orange. Half the Petri dishes (all of which had been strongly heated in the oven) had blotting-paper in their lids. This had been kept wet for seven days before the plant tissue was put in. The other half of the Petri dishes were without

blotting-paper. Eight Petri dishes were allotted to each plant, and eight were set up as controls, each lot being subdivided as follows :

- 2 with B.P. and containing watch-glass of water.
- 2 " " " dil. NaOH.
- 2 without B.P. and containing watch-glass of water.
- 2 " " " dil. NaOH.

In each dish were two slides, each with two drops of the same spore suspension. There were thus eight drops under each treatment. After two days the state of germination was determined. In all cases no definite difference could be seen or measured between the germinations over water and the corresponding ones over dilute caustic soda, thus again showing that carbon dioxide is not the factor concerned. Each figure in the table represents the average of twenty-five spores taken from one drop, and each set of twenty-five measurements was taken from different drops. These figures will also serve to illustrate the degree of uniformity met with in these measurements.

TABLE V.

<i>Experimental condition.</i>	<i>Amount of germination after 2 days.</i>	<i>Average.</i>
Without blotting-paper.		
Control	2.12, 1.60, 2.08, 1.83, } 1.50, 1.72, 1.56, 1.60 }	1.76
Apple	3.44, 3.16, 3.12, 3.24	3.24
Orange	Inhibition in all cases ¹	0.00
Eucalyptus	5.84	5.84
Potato	0.44 ²	0.44
With blotting-paper		
Control	0.14, 0.10, 0.14, 0.16,	0.14
Apple	2.96, 2.92, 2.72, 2.92	2.88
Orange	Inhibition in all cases ¹	0.00
Eucalyptus	3.16, 2.88	3.02
Potato	Almost completely inhibited	0.0 +

This experiment shows clearly the following points :

(1) That *Botrytis* spores are distinctly stimulated by the presence of apple tissue and of *Eucalyptus* leaves.

(2) That orange rind and potato tubers produce equally distinct depression of germination.

✓ (3) That the presence of wet blotting-paper depresses germination.

Again it may be noted that the stimulating effect is best seen in the series where the control germination is feeble (i.e. when blotting-paper is present), whereas the retarding effects show best in the series where blotting-paper is absent and where accordingly the control germination is good.

Though the spores of *Botrytis cinerea* were found to be the most

¹ Incipient germination on 4th day.

² This was the best germination seen in any of the eight drops.

suitable objects for this work, similar results were also obtained with the spores of other fungi, as is shown in Table VI.

TABLE VI.

Fungus.	With blotting-paper.		Without blotting-paper.	
	Control.	Apple.	Control.	Apple.
<i>Botrytis cinerea</i>	0.0 +	3.52	1.38	4.12
<i>B. parasitica</i>	0.44	1.06	1.52	1.14
<i>Fusarium sp.</i>	0.00	2.16	2.00	2.04

In the case of the other fungi tested in this experiment, measurements were not taken, but the following is a description of the results:

Penicillium glaucum: results follow the same lines as with *Botrytis cinerea*, i. e. apple without B.P. > apple with B.P., > control without B.P., > control with B.P.

Colletotrichum Lindemuthianum: a small amount of germination in the control without blotting-paper: none at all in any of the others.

Monilia fructigena: with young spores the amount of germination in all cases was so great that measurement was impracticable: with older spores the amount of individual variation in germinative capacity is very great. This fungus appears to be very unsuited to work of this description.

The results with *B. parasitica* require further description. The figures in the above table give a very inadequate representation of the picture presented. All the germinations in presence of apple show stout germ-tubes, whereas the latter are very slender in the controls. The best illustrations of this effect have been met with in the case of this fungus. With *Fusarium* spores, a distinct stimulation by apple was only seen in the presence of blotting-paper. This fungus is also not very convenient for measurement, as it is generally not easy to decide where the germ-tube begins and the spore ends. The result with *Colletotrichum* is noteworthy as indicating the opposite response to the presence of apple tissue.

It is interesting also to note that with the exception of *Monilia* (for which no data were obtained) all the fungi tested reacted in the usual way to the presence of blotting-paper.

The effect of plant distillates and of various chemical substances was also tested.

Leaves of *Ruta* and of *Pelargonium* were placed in water in a retort and subjected to distillation. The distillates were then tested either by adding one drop of each to one drop of a spore suspension or by placing filter-paper wetted with the distillates in the Petri dishes. The control series had water in place of the distillate. The amount of germination in the various distillate preparations varied from four to ten times that in the controls. There is no doubt, therefore, as to the effects produced by the plant tissues being due to volatile substances arising from the latter.

A number of pure chemical substances were also tested, including various esters of ethyl alcohol and several essential oils. The following is an experiment with ethyl acetate: In each of four 3-litre containers 50 c.c. of water was placed and in order 0, 1, 2, and 10 drops of ethyl acetate added. The slides with spore drops were then put in and the lids of the containers immediately replaced. After two days the following figures for germination were obtained:

Control.	1 drop Eth. Ac.	2 drops Eth. Ac.	10 drops Eth. Ac.
0.36	1.82	1.54	0.46

These results indicate a distinct optimum effect, as was to be expected.

A rough comparison was made of several ethyl esters by testing the effect of a few c.c. of a saturated watery solution of each and of a tenth dilution of the same on the germination of *Botrytis* in Petri dishes. In all cases inhibition was shown in the presence of the saturated solutions, while stimulation appeared over the tenth-saturated solutions of ethyl acetate, malate, and citrate. In presence of even the dilute solution of ethyl oxalate, complete inhibition was still shown.

Any further comparison was not attempted, as obviously that would require a long investigation of itself. Nevertheless, these experiments show distinctly that the effects recorded earlier in this paper for plant tissues can be paralleled by the use of volatile organic reagents alone.

As good stimulation had been obtained with leaves of *Eucalyptus* and *Pelargonium*, tests were made with the oils of eucalyptus and geranium, chiefly with the former. Though several experiments were made with different amounts of oil, nothing in the nature of stimulation has yet been seen with either of these oils. Even one drop of oil in a large 3-litre vessel is sufficient to inhibit spore germination. With water that has been shaken up with the oil and then diluted to various degrees, no definite stimulation has been seen at any point. It may of course be difficult to arrive at the suitable concentration, as the stimulative effect might only obtain over a very narrow range of concentration, but it is clear that with oil of eucalyptus stimulation is difficult to obtain, if it can be obtained at all, whereas good stimulation has always been obtained with *Eucalyptus* leaves, as witness the experiment quoted on p. 293. It is not, of course, at all certain that the stimulating principle in *Eucalyptus* leaves is the so-called oil of eucalyptus at all. It may quite well be some other volatile substance present in the plant.

Oil of cloves was also tested, and it appeared that when present in very small amount it was highly toxic to fungal spores.

A fuller account will now be given of the blotting-paper effect which has already been several times mentioned. The magnitude of this effect

may be seen from several of the tables quoted above. It is shown equally well by blotting-paper and by the ordinary filter-paper. The method adopted to get the effect was simply to place discs of blotting- or filter-paper in the usual way in the lids of Petri dishes, to wet the papers with distilled or tap water, and to allow them to remain wet for a week. Control experiments showed that the effect began to appear in three or four days. On comparing a series of dishes in which the paper had remained wet for seven days with another series in which the paper was left dry for these seven days and was only wetted at the time the germination test was begun, it was found that the latter gave germination equal to that given in the absence of the paper, whereas the former showed reduced germination. A certain latent period is thus required for production of the effect. The effect is also shown as markedly when the wet paper is placed in the bottom instead of in the lid of the Petri dish, and is thus not due to interference by the paper with the conditions of aeration in the dish.

The method of producing the effect being thus known, experiments were carried out to determine in what ways it could be removed. The earlier work gave a certain amount of indication that the effect was produced more strongly in light than in darkness, and thus a photochemical action of light on the paper was suggested, resulting in the formation of some volatile substance, such as perhaps hydrogen peroxide or formaldehyde. Later experiments, however, have shown that the effect is obtained in much the same degree with wet blotting-paper in the dark, and all the results obtained indicate that the action is due to some organism or organisms growing in the moist paper. Thus after any treatment which would destroy organisms present in the paper the retarding effect does not appear. The following treatments have been shown to be effective in preventing the appearance of a retarding action: Sterilizing the paper in a moist condition (dry air sterilization of the paper is hardly allowable as this process much reduces its hygroscopic capacity); placing a few drops of chloroform in the Petri dishes (all traces of chloroform were found to have disappeared at the end of the incubation period); wetting the paper with dilute caustic soda, dilute copper sulphate, or potassium permanganate solution. In paper which is showing the retarding effect, this can be removed by washing with alcohol, followed by thorough rinsing with distilled water, or simply by the addition to the paper of a few drops of dilute permanganate solution. The effect thus appears to be due to biotic causes.

No attempt has been made to isolate the organism or organisms concerned, and thereby to produce the effects with pure cultures on the paper; nor has the volatile substance been determined further than the experimental proof that it is not hydrogen peroxide. This was carried out as follows: Various dilutions of a 20-volume solution of hydrogen peroxide

were added to the blotting-papers in a series of Petri dishes, by which means it was established that at a dilution of 1 in 100 less retardation was caused than with blotting-papers which had been left wet with water for a week. At the end of the germination test, the addition of permanganate solution showed no definite reaction for hydrogen peroxide in the latter case, whereas a distinct rapid decoloration was shown in the former. The retarding effect on spore germination cannot thus be due to hydrogen peroxide.

The retarding effect of blotting-paper is not demonstrable when small traces of nutrient are present in the germination drop. The following test of germinations in various dilutions of turnip extract, carried out in Petri dishes with and without blotting-paper, illustrates this point :

TABLE VII.

	<i>T.E./1,000.</i>	<i>T.E./5,000.</i>	<i>T.E./25,000.</i>	<i>T.E./100,000</i>	<i>Water.</i>
No B.P.	long	3.76	3.12	2.88	2.52
B.P.	long	4.12	0.74	0.20	0.20

The effect, therefore, is only demonstrable in sowings in water or in extremely dilute nutrient.

DISCUSSION.

The experiments described in the present paper point to a marked degree of sensitiveness on the part of fungal spores to the presence of traces of volatile organic substances in the atmosphere to which the spores are exposed during germination. Such stimulation is not large absolutely, and accordingly is only in general demonstrable when the spores are placed in conditions of very limited food supply, as for example in pure water. The behaviour of fungal spores in water is a matter of considerable importance in the study of pathology. The bearing of the results of this paper on such study may now be indicated.

Germination studies are in many cases carried out in an incubator, and usually in the presence of other fungal cultures. Every one who has worked with fungi knows the characteristic 'fungous' odour which clings to an incubator which is in constant use. In the case of germination studies in which the ordinary cultural media are used, any effect arising from the atmosphere would probably be negligible; when, on the other hand, one is dealing with germinations in water it is not at all certain that the effects produced by these volatile substances could be ignored. As a case in point, we may adduce the effects produced by blotting-paper already described. Here there seems no doubt that the effects in question are produced by the action of some organisms growing on the moist paper and giving off some volatile organic substance deleterious to germination.

In carrying out studies of germination in water, one should therefore

take steps to reduce disturbing atmospheric factors to a minimum. In cases where relative effects only were being considered, any disturbance arising from volatile substances would probably be of less consequence, but if it was sought to determine the absolute amount of germination, the result obtained, e.g. in the presence of a large number of other cultures, would in all probability not represent the intrinsic germinating capacity of the fungus under investigation. In particular the custom of placing moist paper in the lids of Petri dishes in accurate studies of germination is not to be recommended unless proper control measures are taken. Furthermore, one may ask in this connexion how far the failure, so often reported, to obtain vigorous attack under laboratory conditions with organisms which readily produce attack under field conditions may not be due to some such influences as are here indicated.

The possible significance of the results of the present paper for the physiological analysis of pathological problems will now be discussed: (1) in relation to the conditions in the infection drop, (2) in relation to growth of the fungus after penetration of the host.

It was shown in earlier papers of this series that the conditions present in the infection drop are in general those of feeble nutrition. The vigour of germination in the infection drop—a factor of primary importance in deciding whether attack is possible or not—has been shown in No. VIII of the present series to be influenced by the passive exosmosis of food substances from the host tissue into the infection drop. The present results show that, granted suitable conditions, a like effect can be produced through the action of volatile substances arising from the plant and accumulating in the atmosphere. *A priori* one would expect this latter effect to be least in the open field. On the other hand, one would anticipate that it would play a greater part under conditions of storage. Here the atmosphere is stagnant and volatile substances can accumulate to a degree comparable with the conditions which gave results in the present experiments. To cite particular instances, one may confidently state that the atmosphere of an apple store is very favourable to *Botrytis* germination; and that the converse is true for the atmosphere of a potato store.

As regards the effect of volatile constituents of the plant on the growth of the fungus, once the latter is inside, little can be said at present. Unpublished experiments have clearly shown that the capacity of crude unboiled plant extracts to cause germination and growth of fungal spores is in many cases very different from that of the boiled extracts. An extreme case of this is afforded by onion juice, which inhibits *Botrytis* germination when in the unboiled condition, whereas when boiled it allows ready germination and growth. An investigation of this phenomenon is in progress and, though it is fairly clear that other factors also are concerned, the influence of volatile elements is being kept in view.

The question of a specific action on a particular fungus of the volatile substances from a particular plant has scarcely been touched. This is a problem of considerable experimental difficulty, and as such it has been decided not to follow it up for the present. The difficulty in question is one of special methods rendered necessary by the nature of the problem. The active substances are volatile and produce their effects when present in small quantity. A quantitative examination would therefore involve considerable technical difficulties. As a further difficulty one would have to reckon with the strong probability that the curve expressing stimulation in terms of concentration would show a pronounced optimum, so that in practice one would not be certain, short of considerable experimentation, whether a small effect in any particular case was due to too much or too little of the stimulant. The interpretation of the result in any particular experiment would thus not be easy, especially as at the present there seems to be no ready means of regulating or measuring the concentration of the stimulant. As an illustration of this difficulty we may refer to the experiment quoted on p. 294. The presence of apple tissue was there seen to produce distinct stimulation of *Botrytis cinerea* spores, but to inhibit spores of *Colletotrichum*. There would appear to be a certain appositeness in this result—that *Botrytis* spores are stimulated by volatile substances arising from apple fruit, which is one of its natural hosts, whereas *Colletotrichum*, which does not occur on apple, is not so stimulated. But this conclusion would be premature, as it is quite possible that under suitable conditions the spores of the latter fungus could also be stimulated by the presence of apple tissue. Obviously a considerable amount of work is still required in this connexion.

SUMMARY.

1. The germination of *Botrytis cinerea* spores is increased by the action of volatile substances arising from certain plant tissues, such as apple leaves and fruit, leaves of *Ruta*, *Eucalyptus*, &c.
 2. In other cases reduced germination or even inhibition is produced—viz. with tissue of potato tuber, onion leaves or bulb scales.
 3. Reduced spore germination due to volatile substances produced by growth of organisms in wet filter- or blotting-paper is described.
 4. Similar stimulating and retarding effects can be produced by the action of simple chemical substances such as ethyl acetate.
 5. A number of other fungi were tested in these respects and found to behave similarly to *Botrytis cinerea*.
 6. The bearing of these results on general mycological technique and on problems of physiological parasitism is discussed.
-

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A Note on Conjugation in *Zygnema*.

BY

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With Plate XII and two Figures in the Text.

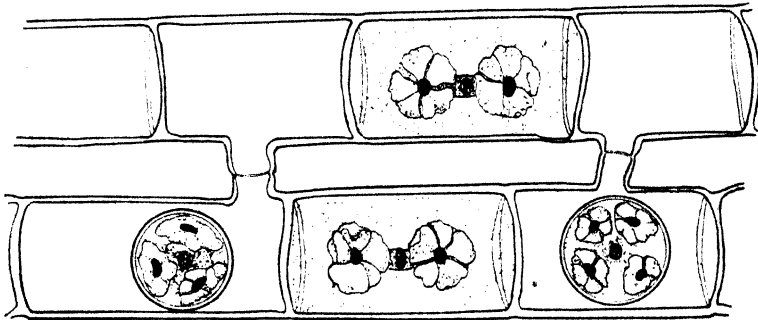
MATERIAL. Certain preserved material of *Zygnema*, which was known to have been in the possession of the Department for a number of years, although it was unfortunately impossible to discover its source, was found on examination to show abundance of zygospores. As conjugation in *Zygnema* is rare in nature, and has not hitherto been induced experimentally, it was felt that a preliminary account of the stages shown in this material would be of interest. In order to confirm the observations on the structure of the chloroplast in the fixed material, fresh material of another species (showing only the vegetative stage) was also studied. The resemblance in general character of the chloroplasts in the two species was very striking.

Treatment. The preserved material was in 70 per cent. alcohol, but as an additional precaution was fixed for 24 hours in dilute chromacetic acid. It was then placed in 5 per cent. glycerine, which was concentrated until of the thickness of pure glycerine. The material was then stained in safranin and aniline blue, and mounted in Venetian turpentine. Material thus treated showed the nucleus stained pink, the chloroplasts light blue, while the pyrenoid took a very dark blue. The cytoplasm remained practically colourless. Paraffin sections $6\ \mu$ thick were also cut, and stained with Heidenhain's iron-alum haematoxylin, but owing to the small size of the nucleus and the originally imperfect fixation, no details of nuclear structure were made out.

The fresh material was studied in water and iodine mounts: the latter reagent brought out very clearly the structure of the chloroplast and the pyrenoid. *

Description. By comparison with herbarium material at the Natural History Museum, the conjugating material was identified as *Zygnema stellinum*, (Vauch.) Ag. The fresh material corresponded to *Z. cruciatum*.

Zygnema stellinum: cells of filament sub-cylindrical $30-35$ (33) μ in diameter, $70-80$ μ in length. The cell-wall is thin cellulose, with no signs of lamellation even when treated with caustic potash. The cytoplasm is somewhat vacuolated, with an apparently denser median strand (zugon), in which the nucleus is suspended. The nucleus is small (3 μ), sub-spherical, with well-marked membrane and nucleolus. No details of nuclear division were observed. In the vegetative cell there are two elaborate chloroplasts, suspended in a small amount of cytoplasm in the centre of the cell. Each plastid contains a single large (3 μ) pyrenoid, which is surrounded by a more or less diffuse starch zone. The exact method of starch-formation was not determined. The plastid is never apparently seen side-on, as it is in *Mougeotia*, so that it would seem to be a radially organized body. It appears (in the fresh material) as a complex mass of much-dissected fronds, radiating from and incurved over the central pyrenoid (see Plate XII, Fig. 1).



TEXT-FIG. 1. *Zygnema stellinum*, showing scalariform type of conjugation. ($\times 500$ times.)

In the fixed material the dissection was not quite so marked, due possibly to shrinkage during manipulation.

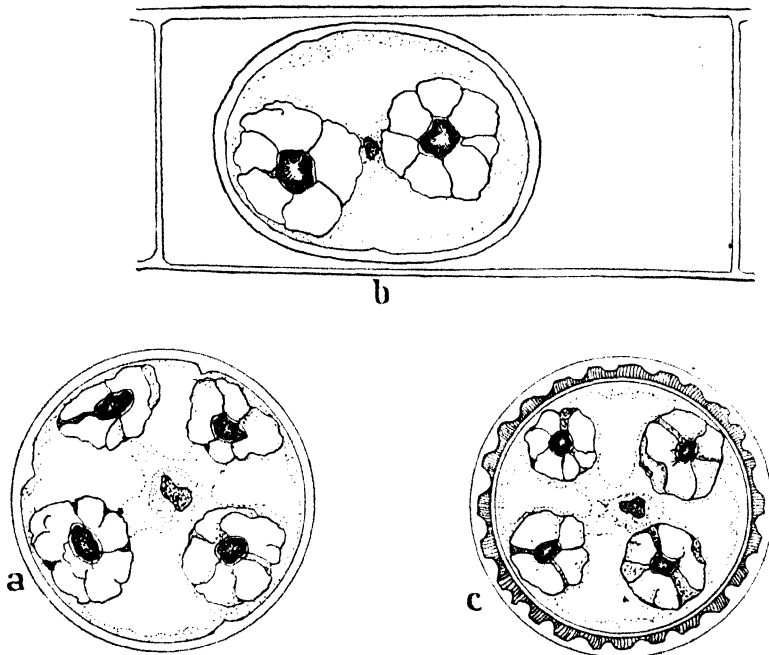
Conjugation. The material at hand showed abundance of zygospores, but no earlier stages in the process of conjugation. Conjugation was by the scalariform method, the zygospore being lodged in one or other of the conjugating cells, which is not inflated or distorted in any way (Text-fig. 1). 'Cross'-conjugation was not observed, but there were numerous cases of differentiation of sex in a single filament, as has been frequently reported in *Spirogyra* (Cunningham (1)).

The young zygospore is $30-33$ μ in diameter, and approximately spherical. The wall is thin ($1.5-2$ μ), and not yet cuticularized: usually there are four inwardly-directed points on the inner surface. The four chloroplasts derived from the two conjugants remain quite distinct, and retain their characteristic complex shape as seen in the vegetative cell. They are commonly arranged in a tetrahedral fashion (see Plate XII, Fig. 2).

The nucleus is approximately double the size of the vegetative nucleus ($7-8\ \mu$), and frequently appears constricted. Whether this appearance is due to incomplete fusion or incipient division was not determined.

The cytoplasm is much vacuolated, with many highly refractive droplets which dissolve completely in absolute alcohol or ether, and so would seem to be of an oily nature (Text-fig. 2, *a*).

Occasional azygospores are found, which are more ovoid, and contain



TEXT-FIG. 2. *a*. Young zygospore of *Z. stellinum*, showing four chloroplasts and fusion nucleus in centre of spore. *b*. Young azygospore, ovoid, with only two chloroplasts. *c*. Mature zygospore, showing sculpturing of the mesosporium: the four chloroplasts remaining distinct. (All $\times 1,300$.)

only two plastids. The nucleus also is no larger than the vegetative nucleus (Text-fig. 2, *b*).

The mature zygospore (Text-fig. 2, *c*) shows sculpturing of the mesosporium into blunt bosses, which are yellow-brown and cuticularized. The four chloroplasts retain their individuality: even at this stage there is no sign of any fusion or division of the plastids. The four tetrahedrally arranged ingrowths on the wall may indicate a subsequent tetrad division, similar to that described in *Spirogyra* (Tröndle (2)), for the first division of the zygote, but this point must be left for further observation to decide.

SUMMARY.

Zygnema stellinum shows scalariform conjugation, with the zygospore lodged in one or other of the conjugating cells.

The vegetative cell contains two complex chloroplasts, consisting of much-dissected fronds, incurved over a central pyrenoid. The nucleus is suspended in a strand of denser cytoplasm in the centre of the cell.

The young zygospore is spherical, with a thin wall and four chloroplasts. The fusion nucleus is in the centre of the spore, and is twice the size of the vegetative nucleus and often constricted.

Occasional azygospores are found, which are more ovoid and have only two chloroplasts.

The mature zygospore shows sculpturing of the mesosporium, which is yellow-brown and cuticularized. The four chloroplasts remain distinct even at this stage.

In conclusion, I wish to express my thanks to Professor R. R. Gates, at whose instigation the work was undertaken, for his constant advice and criticism during the course of the investigation; also to Mr. Gepp, of the Natural History Museum, for his kind assistance in identifying the species.

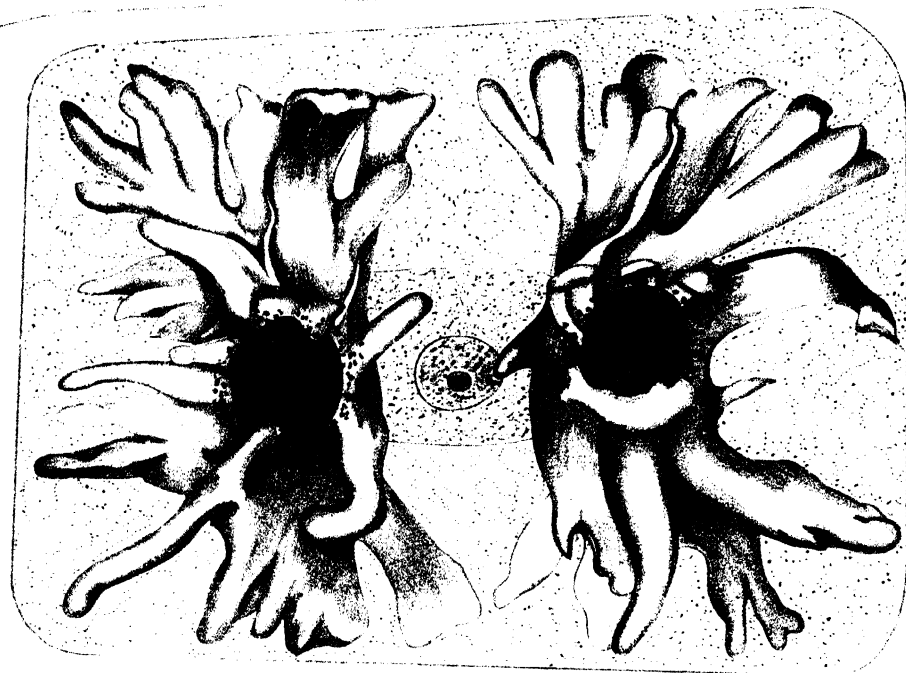
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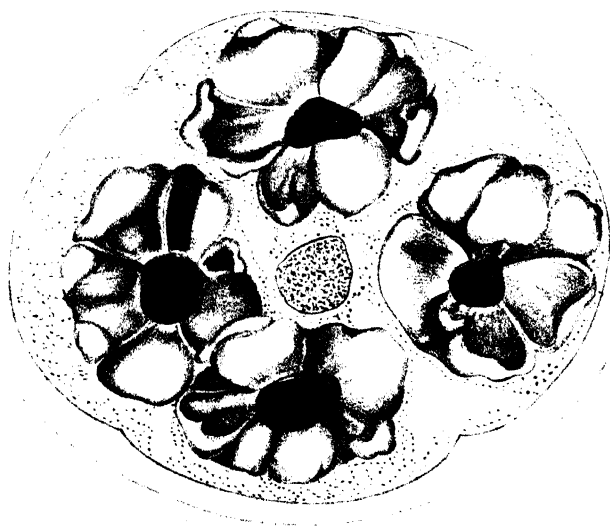
EXPLANATION OF PLATE XII.

Fig. 1. Vegetative cell of *Zygnema cruciatum*, showing two complex chloroplasts, each with one pyrenoid, nucleus suspended in centre of cell in a strand of denser cytoplasm.

Fig. 2. Young zygospore of *Z. stellinum*, showing four chloroplasts, less dissected than those of the vegetative cell. (Both figures $\times 2,300$.)



1.



2.

Ruth lith et imp

SMITH-ZYGNEMA.

Further Studies of the 'Brown Rot' Fungi.

I. A Shoot-Wilt and Canker of Plum Trees caused by *Sclerotinia cinerea*.

BY

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With Plates XIII and XIV.

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I. GENERAL OBSERVATIONS.

ABOUT the middle of May 1919 a row of Victoria plum trees in the fruit plantation at Wye College was examined for the early symptoms of 'Wither-Tip', a disease from which some of the trees had suffered in previous years. This disease, described fully elsewhere,¹ is characterized, as the name implies, by a withering of the tips of the young green terminal shoots of the branches. In the present case the terminal shoots had made but little growth at the time the examination was made and typical instances of Wither-Tip were not seen. It was found, however, that a number of short leafy shoots had been recently killed; these shoots were borne laterally on the twigs produced the previous year, and they were such as would have remained short and become 'fruiting spurs' in the following season.² The largest leaves on these wilted shoots were only from 2 to

¹ Wormald, H. : A 'Wither-Tip' of Plum Trees. Ann. Appl. Biol., v, 1918, pp. 28-59.

² Each of these lateral shoots consists of a number of leaves borne on a very short axis; the buds which develop in the axils of these leaves give rise to leaves and flowers the following spring (Plate XIII, Fig. 6).

3 cm. in length, and the wilt, therefore, must have occurred soon after the buds expanded (Plate XIII, Fig. 1).

Usually only one wilted shoot was to be found on a twig, but sometimes two and occasionally three were found on one twig. They were most numerous on trees which had been infected with the 'Wither-Tip' disease during the previous year; the withered tips had persisted through the winter, and during May bore numerous powdery tufts of *Monilia cinerea*, the conidial stage of *Sclerotinia cinerea*, (Bon.) Schröter. The wilted shoots were, as a rule, in close proximity to the dead tips, those shoots immediately below such tips being particularly subject to attack. It was suspected, therefore, that the wilt was caused by *S. cinerea*, the withered tips of the previous year's shoots serving, in all probability, as the principal source of infection, at any rate in this particular instance, since mummied fruit was absent from most of the trees (a result of the almost total failure of the crop in 1918), while a few of the trees bore one or two mummies only. The weather had been wet and rather cold while the buds were expanding, and the damp atmosphere probably favoured infection and also induced free development of conidia on the pustules of the withered shoots.

During the first week of June it was noticed that cankers had developed round those nodes where the wilted shoots were inserted. In a few cases the canker had girdled the twig and so caused a withering of that portion distal to the canker (Plate XIII, Fig. 5). Some of the cankers which had only partly girdled the twigs were labelled, so that they could be kept under observation to see what rate of progress the cankers made, but from that time onward no further increase in the size of the cankers could be detected, and on cutting cankers across during the first week of July it was found that already callus was developing from the edges of the lesions and tending to cut off the dead bark.

No external signs of a fungus were found on the dead leaves or on the cankers at this stage, except in one case where on one shoot pustules of *Monilia cinerea* were found on the petioles. The almost complete absence of *Monilia* pustules from the leaves was undoubtedly due to the dry weather which generally prevailed about this time.¹ When, however, infected shoots were placed in a moist atmosphere at room temperature (about 18° C.) *Monilia* fructifications appeared on the dead leaves within twenty-four hours.

The cankers were seen as slightly sunken areas, approximately elliptical in surface view, being broadest at the node, but, as a rule, they extended downwards to a greater distance than upwards. Thus of seventeen cankers which were measured two showed an upward extension equal to the downward, while in the rest the downward extension was the greater. The

¹ The pustules seen on the petioles were discovered on a day following a showery evening.

cankers examined were on twigs 3 to 7 mm. in diameter, and in most cases had extended laterally about half-way round the twigs; one of the largest of these was 4.4 cm. in length, extending from the node 1.8 cm. upwards and 2.6 cm. downwards.

The infected tissues undergo a disintegration resulting in gummosis, the gum frequently being so copious as to exude in drops (Plate XIII, Fig. 2). A characteristic feature of these lesions is a necrosis of the young xylem elements far in advance of the fungal hyphae. Mycelium is to be found only in the tissues of infected shoots and in the cankers, while disintegration of the xylem elements can be traced for several centimetres (as far as 10 cm. in one case) from the cankers. On cutting a twig transversely at a short distance above or below a canker the disintegrated xylem is seen as a row of dark dots, just visible to the naked eye and easily seen with a pocket lens (see Plate XIV, Fig. 10), forming an arc parallel with, and about 0.5 mm. from, the cambium layer, and situated on the same side of the twig as the canker. Microscopic examination resolves the dots as gum 'pockets' in the xylem. In longitudinal radial section through a cankered node the necrosis is seen as a dark line extending upwards, and downwards from the canker and again parallel with the line that indicates the position of the cambium layer (Figs. 8 and 9). It was present even when there was no visible canker at the node apart from the base of the withered shoot itself. As with the cankers themselves, this necrosis of the xylem extends, as a rule, farther below the node than above it.

Observations on the extension of the gum 'pockets' from the original lesion were recorded in four cases, as follows:

Description of the original lesion.	Extension of gummosis (xylem necrosis) from the original lesion.	
	Upwards.	Downwards.
1. No canker visible on outside. Shoot dead and tissues brown as far as and including xylem formed in the current year. Hyphae found in the shoot only.	2.1 cm.	1.5 cm.
2. No canker visible on outside. The inner basal tissues of the shoot not brown; the younger tissues of the shoot brown and containing hyphae.	0.3 cm.	1.5 cm.
3. Canker present, extending two-thirds round the twig and upwards from the node for 1.6 cm., downwards for 2.4 cm.	5.5 cm.	8.5 cm.
4. Canker present, two-thirds round the twig, extending upwards from the node for 1.3 cm., downwards for 1.8 cm.	5.0 cm.	10.0 cm.

No organism has been found in these disintegrated tissues beyond the cankers, and the necrosis appears to be brought about by an enzyme (secreted by the fungus) which diffuses along the young vessels and causes their hydrolysis. Whatever may be the cause, the action ceases early in the growing season, for if, in the winter following infection, a twig is cut across at 2 or 3 cm. above or below a canker, the disintegrated vessels are found to be embedded in the wood and confined to the inner edge of the ring of

xylem produced during the season in which infection occurred (Plate XIV, Fig. 11).

Although pustules of *Monilia cinerea* may develop on the dead leaves during wet weather in the summer, they are not found on the bark of the shoots or on the cankers until winter approaches. Early in December *Monilia* fructifications were seen on the bark of a number of the dead shoots and on the petioles of the withered leaves. Conidial fructifications continue to develop during the winter months, and in February nearly all the cankers labelled in June of the previous year bore grey conidial tufts, usually at the base of the short shoots, but also frequently on the bark of the cankers. The cankers by this time were, in many cases, nearly covered with callus, which, developing from the two sides, had almost reached the middle line and had caused the bark to become ruptured (Plate XIII, Figs. 3 and 4).

The conidia produced on the *Monilia* pustules of the cankers during winter and spring have dimensions similar to those of *M. cinerea* found on the mummied fruit and on withered tips at that time of the year. Conidia taken from a pustule growing from a canker in March showed variation in size from $7.5 \times 5.5 \mu$ to $16 \times 11.5 \mu$; they were mostly, however, within the range $10-12.5 \times 7-9.5 \mu$, and the average of 100 conidia was $11.3 \times 8.4 \mu$.

The conidia produced on the young leaves in summer are larger than those of the cankers, their size being of the same order as that of the 'summer conidia' of the fruit.¹

Although the wilting of the short shoots of the plum trees had not been observed previous to the spring of 1919, or if noticed had not been recognized as a 'Brown Rot' disease, there was evidence that it had occurred during the seasons 1918 and 1917 on some of the trees examined, for certain cankers were found (in 1919) which from their condition and their position on the branches indicated that infection had taken place through the short shoots. Thus on two-year-old wood there were cankers at nodes where there must have been short leafy shoots during the previous year. These cankers were partly covered by callus and almost invariably bore pustules on the short dead shoots or on the cankers; they correspond to, and were similar to, those cankers which were kept under observation and found to produce pustules during the season subsequent to infection. Somewhat similar cankers, but with callus further developed and bearing no pustules (most of the dead bark having peeled away), were found on the three-year-old wood; these were evidently a further stage in the formation and the healing over of the Shoot-Wilt cankers (Plate XIV, Fig. 7).

II. ISOLATION AND CULTURAL EXPERIMENTS.

Pure cultures of *Sclerotinia cinerea* could easily be obtained from the mycelium found in the tissues of the young cankers. The surface of

¹ See p. 315.

a canker was first cleansed by wiping it with cotton-wool moistened with 95 per cent. alcohol; transverse sections were then made through the cankered portion and the sections placed in sterile water in a flamed watch-glass.¹ As a further precaution against contamination the outer layers of the bark were teased away with flamed needles, and particles of the internal brown tissues (bark and wood) were removed to a second watch-glass of water, from which they were transferred to carrot agar or prune agar in Petri dishes. In such plates, kept at room temperature (about 18° C.), the hyphae grew out readily and within six days had given rise to discs of mycelium 2.1 to 2.5 cm. in diameter; at this stage further primary growth was checked, but fan-shaped lobes appeared at certain points on the margin, and these gave rise to a zone of mycelium around the primary growth, and later another zone developed in a similar way (Plate XIV, Fig. 13). This mode of growth in agar plates is also shown by cultures of *Sclerotinia cinerea* when obtained directly from ascospores, and, as pointed out in previous papers,² is a character which distinguishes this fungus, as found in Britain, not only from *S. fructigena*, but also from the Brown Rot fungus which is common in America.

Cultures obtained in this way in June and July were almost invariably pure, so far as could be seen. For further experimental work it was considered desirable, however, to obtain cultures derived from single conidia. The agar-plate cultures were quite barren, but on transferring a little of the mycelium to sterilized potato in tubes, grey tufts of conidiophores with their chains of conidia appeared within a week. Conidia taken from the cultures on potato were isolated³ on agar plates and the resulting 'sporelings' gave pure line cultures.

In winter pure line cultures were obtained direct from conidia taken from a canker; in general habit such cultures resembled those obtained from the barren mycelium of the young cankers.

The cultures of the Shoot-Wilt fungus on sterilized potato were typical of *Sclerotinia cinerea* f. *pruni*, conidia being produced more freely than is the case in potato cultures of *S. cinerea* f. *mali*. These conidia were larger than the 'winter conidia' produced on the cankers, and were of the same order as those produced on the leaves and fruit in summer, the average size being approximately $17 \times 12 \mu$.

¹ Two watch-glasses were sterilized simultaneously, by passing them several times through a Bunsen flame, and left to cool with one inverted over the other to eliminate as far as possible contamination by the entrance of spores floating in the air.

² Ann. Bot., xxxiv, p. 164; xxxv, 1921, p. 129.

³ For details of the method adopted by the author when isolating *Monilia* conidia on agar plates see Ann. Bot., xxxiii, pp. 371-2.

III. INOCULATION EXPERIMENTS.

Using pure cultures of the fungus, inoculation experiments were carried out as follows:

(a) Inoculation of plum leaves on short shoots to confirm the evidence supplied by observations in the open that the conidia of *Sclerotinia cinerea* are able to cause infection of the leaves and produce cankers on the twigs by invasion from the infected leaves.

(b) Inoculation of plums (fruit) to ascertain whether the fungus, when raised from the small 'winter conidia', produces typical 'summer conidia' when grown on the fruit, as was shown to be the case in the 'Wither-Tip' disease.

(c) Inoculation of apple flowers to determine whether the form causing this Shoot-Wilt and canker of plum trees is identical with the form causing the 'Blossom-Wilt and Canker' disease of apple trees (forma *mali*) or with the form commonly occurring on plum and cherry trees (forma *pruni*), the latter in a considerable number of inoculations having invariably failed to induce Blossom-Wilt of apple trees.

(a) *Inoculation Experiment on Plum Leaves.**Experiment 1.*

Young shoots of Victoria plum trees growing in pots in the greenhouse were used in this experiment. Two series of inoculations were made on April 1, 1920, viz.:

(a) Five of the shoots were sprayed with distilled water; one leaf on each shoot was then punctured (four punctures in a group between the midrib and the margin of the leaf) with a sterile needle and the punctured parts inoculated with conidia of the fungus. The conidia had been produced in cultures on steamed potato, and the inoculation was made by taking a particle of the potato on a needle and bringing the conidia-bearing surface in contact with the wounded leaf.

(b) In the second series seven shoots were also sprayed with distilled water, and one leaf on each was inoculated with conidia but without the preliminary puncturing.

One leaf only in each series became infected; notes taken on the rate of progress of the disease in these two cases are as follows:

<i>April 1.</i>	<i>April 14.</i>	<i>April 17.</i>	<i>April 22.</i>
(a) One leaf on each of five shoots inoculated at punctures.	One leaf only infected; a brown discoloration extends to a distance of 8 mm. from the punctures.	The whole leaf is flaccid; a brown area, about 1 cm. wide, extends from near the apex of the leaf into the petiole; the rest of the leaf is green.	Infected leaf withered; the other leaves of the shoot are also wilting.
(b) One leaf on each of seven shoots inoculated without punctures.	One leaf only infected; a brown discoloration extends from the middle of one edge to the base of the lamina.	Petiole and lower parts of lamina brown; apical portion of leaf still green, but flaccid; the other leaves on the shoot are also wilting.	A canker has girdled the twig; the leaves of the terminal portion of the twig are wilting.

Later the infected twigs were cut off and it was found that in both of them the mycelium had invaded the twig from the shoot, since hyphae were seen in the cortex. A drop of gum had oozed out to the surface of one canker. Plate cultures obtained by placing particles of the cortex (three particles taken from each canker) on agar, in the way already described for isolating the fungus from naturally infected cankers, gave rise in every case to typical cultures of *Sclerotinia cinerea*.

In the infected leaf of the first series of inoculations it was seen that the browning began at the punctures. In the leaf of the second series infection started at the edge of the leaf, and there was some doubt as to whether the germ tubes had actually penetrated the uninjured epidermis; the leaf was quite young, its edges being still recurved, at the time of inoculation, and it is possible that, in the act of placing conidia on the lower epidermis, the margin of the leaf may have been slightly injured. This result therefore, particularly as it was the only positive one of that series, cannot be taken as proof that infection can take place on an uninjured leaf. The experiment, however, affords evidence that conidia of *Sclerotinia cinerea* are able to produce infection of plum leaves through small wounds, if not through the uninjured surface, and also that the mycelium in an infected leaf may extend into the shoot and also into the twig bearing the shoot.

Experiment 1, being carried out under greenhouse conditions, eliminated the action of frost as a possible primary cause of Shoot-Wilt.

Experiment 2.

This experiment was carried out on Victoria plum trees in the College plantation in the spring of 1921. Short lateral shoots were labelled and the twigs bearing them were sprayed with sterile distilled water; one leaf on each shoot was inoculated, a leaf which had attained to about half its full size being selected and marked with a loop of cotton tied loosely round the petiole. As in the previous experiment, two series of inoculations were made, (a) in which the leaves were punctured before inoculation, the punctures on each leaf being four in number, about 2 mm. apart, and situated midway between the middle of the midrib and the margin on one side, and (b) in which the leaves were not punctured. Leaves used as controls and for comparison were sprayed, some being punctured, but not inoculated. Six leaves in each series were inoculated on May 3.

In series (b) brown spots or patches were observed on five of the inoculated leaves on May 8, mostly towards the base of the lamina; a few days later these brown portions had fallen out and no further change occurred. It is uncertain whether these were infection spots or not, but a comparison with the results obtained in series (a) and the fact that similar spots were present on comparatively few un-inoculated leaves suggest that they were a result of inoculation; assuming this to be the case, it would

appear that most of the conidia had been washed down towards the base of the lamina, for they had been placed near the middle of each leaf.

In series (a) the punctures showed brown margins in every case on May 8, and on some leaves the punctures were connected by brown cells; in three leaves the discoloration had extended from the punctures to the edge of the leaf on that side of the midrib. Later, further extension of the browning occurred, usually accompanied by a yellowing of the tissues in advance of the browning and by a distortion of the leaf as a result of a check in its development on the inoculated side. Finally, in four leaves the diseased portions dropped out and the rest of the leaf did not become affected; in each of the other two, however, the whole leaf was killed, and in one of them the disease extended into the axis of the shoot, causing the wilting of all the other leaves on the shoot, and then into the twig to form a canker (Plate XIV, Fig. 14). As these are features which have not previously been recorded, details of observation are here tabulated.

Results on six plum leaves punctured and inoculated on May 3.

<i>May 11.</i>	<i>May 16.</i>	<i>May 24.</i>
1. A browning of the tissues extends from the punctures to the margin of the leaf.	The browning extends from the midrib to the margin of the leaf on the punctured side and for a distance of 2 cm. along the edge; there is also a yellowing of the infected side accompanied by distortion.	Leaf withered and brown to base of petiole.
2. A browning extends from the punctures to the edge of the leaf.	The discoloration is now extending towards the midrib; there is a slight yellowing and distortion of the leaf between the punctures and the base of the leaf.	The infected portion has fallen away.
3. There is a brown margin to the punctures.	The browning extends for 2 mm. from the punctures; there is a slight yellowing and distortion on the infected side.	The diseased area, 5 × 6 mm., is now breaking away. [A few days later it had fallen out.]
4. Tissues brown between, and round the margins of, the punctures.	That portion of the leaf from the punctures to the margin is yellowish-green and there is some distortion.	Diseased portion fallen out.
5. The punctures have brown margins; two are connected by brown cells; leaf slightly distorted.	Punctures all connected by brown tissues, leaf distorted, and yellowish between the punctures and the base.	Infected portion fallen out.
6. Punctures connected by brown cells; browning extending to the margin of the leaf.	The browning extends as far as the margin of the leaf and to the midrib, and for 1.5 cm. along the edge; leaf distorted.	Infected leaf withered to base of petiole; other leaves of the shoot are wilting; the lower end of the petiole of one leaf is brown for 6 mm.

Later observations on leaves No. 1 and No. 6, the two which were killed outright, were as follows:

No. 1. On May 31 the tip of the shoot was dead but the lower leaves were alive; by June 10 no further development had occurred except that the infected leaf bore pustules of *Monilia cinerea*.

No. 6. On May 31 the whole shoot was dead and bore withered leaves; pustules of *M. cinerea* were present on the inoculated leaf. By June 4 infection had extended into the twig and there was a globule of gum at the node. On June 15 the twig was removed and photographed (Fig. 14); the canker on the twig was only about 1 cm. in length, but necrosis of the young xylem elements, extending beyond the canker for 0.5 cm. upwards and 4.5 cm. downwards, had taken place as in the case of natural infections. Mycelium was found in the brown tissues of the canker, and particles of transverse sections through the bark placed on agar plates gave rise to typical cultures of *Monilia cinerea*; a subculture, on steamed potato, from one of these produced grey *Monilia* fructifications within five days. Thus, as in Experiment 1, proof was obtained that the fungus used in inoculating the leaf had penetrated into the twig. No mycelium was found in the disintegrated xylem beyond the canker, and thick sections, taken at 1 cm., 2 cm., and 4 cm. below the canker, placed on agar, showed that the fungus was not present, since no growth resulted.

The browning of the tissues bordering the punctures in Experiment 2 was apparently the first external symptom of infection, for the punctures on the control leaves, on May 11 and later, had pale margins; on these uninoculated punctured leaves again there was no distortion, showing that merely puncturing the leaf does not check its growth on the injured side. A comparison with the control leaves therefore shows that infection occurred in all the inoculated punctured leaves of that experiment. The failure of the infection to extend to the base of the leaf even where definite brown areas appeared round the punctures may have been due to (1) the age of the leaves when inoculated, or (2) the very dry weather which prevailed almost throughout the whole period during which observations were made; in all probability both these factors influenced the result. With regard to the age of the leaves it would seem that very young leaves are more susceptible than older ones, since the leaves of shoots showing the wilt, when naturally infected, are almost invariably quite small when killed; this, together with the fact that, in Experiment 2, the one shoot which was eventually killed was almost fully developed when the infection reached the axis of the shoot, suggests that, in order to secure results more comparable with natural infections, the inoculations must be carried out on still younger leaves.

The falling out of the infected tissues in the majority of the leaves suggests that the cells are killed in advance of the hyphae; it is conceivable that such dead cells, rapidly becoming desiccated by the persistent dry condition of the atmosphere, would check further growth of the fungus and, as the uninfected tissues of the young leaf continued to grow, a line of rupture would appear between the dead and the living parts. On the other hand, the mere drying out of the infected tissues would tend to inhibit

the growth of the hyphae by rendering the food-stuffs in the leaf unavailable.

Two other series of inoculations similar to those described under Experiment 2 were carried out at the same time, using a culture derived originally from an ascospore of *Sclerotinia cinerea*. The early stages of infection again appeared, as shown by a brown discoloration round the punctures, together with a yellowing and distortion of the leaf in the neighbourhood of the infected areas, but in no case did infection extend to the base of the leaf, the brown parts eventually falling out.

It is evident from these experiments that the conidia of *Sclerotinia cinerea* are able to infect young Victoria plum leaves through punctures, but it has not yet been established that they can produce infection of uninjured leaves. In severe outbreaks of 'Wither-Tip' the disease is usually associated with aphid attacks; on the other hand, in the wilt of the short shoots there was no evidence of insect injury. The failure to obtain any instances of definite infection through unwounded leaves in these experiments, when observations in the open suggest that such cases occur in natural infections, may be understood when it is realized that the pustules of *Monilia cinerea* on mummied fruit and dead shoots produce conidia throughout the winter and spring, and that the leaves are therefore liable to infection, whenever favourable conditions supervene, from the time the buds open onwards.

An experiment carried out in the laboratory favours the idea that infection of uninjured leaves is possible. Young leaves, on long plum shoots placed with their cut ends in water and kept in a moist atmosphere, were readily infected by placing conidia of *Monilia cinerea*, taken direct from a dead shoot, in drops of water on the uninjured leaves. Brown areas appeared within two days at the inoculated spots, and on the sixth day after inoculation several of the leaves were brown throughout and grey *Monilia* pustules were present on them. This experiment is not conclusive evidence, as the shoots were under very abnormal conditions and the purity of the fungus was questionable; it suggests, however, a line for further research.

(b) *Inoculation of Plums (fruit).*

On July 2, 1919, four plums were inoculated by making a puncture through the skin of each with a sterile needle and inserting conidia taken from a culture of the Shoot-Wilt fungus growing on steamed potato. All became infected and produced grey *Monilia* pustules within a few days. Control plums, punctured but not inoculated, did not become infected.

By July 19 three of the infected plums had fallen, but the fourth was still on the tree and had communicated the rot to two others in contact with it. The dimensions of 100 conidia taken from this plum were found to

range from $10 \times 8 \mu$ to $26 \times 16 \mu$ and $22 \times 18 \mu$, the average being $16.8 \times 12.5 \mu$. These 'summer conidia' on the fruit were thus greater than those conidia produced on the dead shoots and cankers in winter, as will be seen by comparing the dimensions here given with those of the 'winter conidia'.¹

These results confirm those of observations previously recorded,² which go to show that the conidia of *Sclerotinia cinerea* produced in winter, on shoots, cankers, and mummied fruit, are invariably distinctly smaller than those which develop on recently infected fruit, leaves, and flowers in summer.

(c) Inoculation of Apple Flowers.

This experiment was carried out on two trees (variety James Grieve) in the fruit plantation. Inflorescences were selected which bore flowers recently opened, and two flowers were inoculated on each inflorescence. On one tree five inflorescences were inoculated with the Shoot-Wilt *Monilia* and five with the Apple Blossom-Wilt fungus (*Monilia cinerea* f. *mali*); on the second tree three inflorescences were inoculated with the former and three with the latter. Thus each fungus was used to inoculate sixteen flowers on eight inflorescences. The inoculations were made by placing conidia from pure cultures on the stigmas.³

Of the flowers inoculated with the Apple Blossom-Wilt fungus all were killed. On one inflorescence the two inoculated flowers fell off without infection extending into the axis; in the rest, however, the mycelium grew into the spurs and killed all the flowers and leaves on those spurs, the wilting of the leaves being noticeable in from fourteen to seventeen days from the day the flowers were inoculated, a condition typical of the Apple Blossom-Wilt disease.⁴

Of the flowers inoculated with the Shoot-Wilt *Monilia* the preliminary symptoms of infection were observed in an early browning of the styles and a premature withering of the stamens and calyx lobes as seen by comparing the inoculated flowers with normal flowers of the same age. All the inoculated flowers, with the exception of two, fell before setting into fruit, and in no case did the fungus enter the axis of the inflorescence, the other flowers and the leaves showing no signs of infection. In this respect the Shoot-Wilt *Monilia* is biologically similar to isolations of *Sclerotinia cinerea* obtained from *Monilia* fructifications found on plums and cherries, and also to an isolation started from an ascospore when the ascigerous stage was found on mummied plums.⁵

¹ See p. 308.

² Ann. Bot., xxxiv, p. 161.

³ For further details of the method adopted in inoculating flowers with *Monilia* conidia, see Ann. Bot., xxxiii, no. 131, pp. 388 and 390.

⁴ Vide A Blossom-Wilt and Canker of Apple Trees. Ann. Appl. Biol., iii. 159, 1917.

⁵ Vide On the Occurrence in Britain of the Ascigerous Stage of a Brown Rot Fungus. Ann. Bot., xxxv, No. 137, pp. 125-35, Jan. 1921.

IV. COMPARATIVE TESTS FOR PRESENCE OF OXIDASE
IN CULTURES.

In a previous paper¹ it has been shown that two forms of *Monilia cinerea*, referred to as forma *mali* and forma *pruni*, are distinguishable not only by a difference in the degree of parasitism shown by the two forms when apple flowers are inoculated with their conidia, but also by a difference in the rate of secretion of an oxidase when the fungi are grown in liquid culture media. In that article the method adopted in applying the test for the presence of oxidase is given in detail;² the tests as applied to the Shoot-Wilt fungus were carried out as described there, except that another culture medium was used (viz. a 2 per cent. extract of p̄r̄unes) and, the thermostat not being available, the cultures were grown, and the tests carried out, in a warm room at a temperature of about 20° C. instead of at 25° C. as in the previous experiment.

In the present instance two isolations of the Shoot-Wilt fungus were used, and, for comparison, a culture of *Sclerotinia cinerea* originally started from an ascospore, and two cultures of *Monilia cinerea* f. *mali* from apple trees, were tested simultaneously. For convenience these may be indicated by the letters *A*, *B*, *C*, *D*, and *E*, as follows:

- A.* Shoot-Wilt *Monilia*, Isol. I: from the mycelium of a Shoot-Wilt canker.
- B.* Shoot-Wilt *Monilia*, Isol. III: isolation from a conidium of a fructification on a Shoot-Wilt canker.
- C.* *Sclerotinia cinerea*, Isol. I: from an ascospore of an apothecium found on a mummied plum.
- D.* *Monilia cinerea* f. *mali*, Isol. XXIII: from a conidium of a Brown Rot canker on an apple tree (Kent).
- E.* *Monilia cinerea* f. *mali*, Isol. XXIV: from the mycelium in a dead spur of an apple tree (Ross-shire).

B and *E* were the fungi used in the experiments described under 'Inoculation of Apple Flowers'; *E* produced typical Apple Blossom-Wilt, while *B* failed to do so.

Two plate cultures of each were started, and when they were ten days old five of them (viz. one of each isolation) were tested for oxidase, guaiacum and pyrogallic acid (2 per cent. solution) being the reagents used. By the tenth day the mycelium of *D* and *E* was much darker than that of *A*, *B*, and *C*. The liquid was strained off from the mycelium, the former only being used in the tests.

D and *E* readily gave the oxidase reaction, a blue colour being evident in the guaiacum tubes within half an hour; the colour developed into

¹ Wormald, H.: The 'Brown Rot' Diseases of Fruit Trees. II. Ann. Bot., xxxiv, 1920, pp. 143-71.

² loc. cit., pp. 147-50.

a bright blue within the next half-hour, and later it was a still deeper blue. *C* gave a trace of colour at the end of two hours, *A* and *B* not until three hours. The colour gradually became a little deeper in tone, but it was still a pale blue in the tubes of *A*, *B*, and *C* at the end of twenty-four hours, the reaction being a little more pronounced with *C* than with *A* or *B*. A corresponding yellowing appeared in the tubes containing pyrogalllic acid, the colour again being more intense with *D* and *E* than with *A*, *B*, and *C*.

On the following day the other five cultures were similarly tested, with the same general result, *D* and *E* readily giving the oxidase reaction with guaiacum and with pyrogalllic acid, the rest giving a comparatively feeble reaction; *C*, however, again being a little more active than *A* or *B*.

None of the isolations used in this experiment had been previously tested for the oxidase reaction, so that the result is further evidence in support of the conclusion previously arrived at, that the forms *mali* and *pruni* can be distinguished in the laboratory by applying comparative tests for secretion of oxidase in liquid culture media. In this connexion the fact that the two isolations of forma *mali* used in the above experiment were obtained from specimens received from such widely separated counties as Kent and Ross-shire is not without interest.

V. 'SHOOT-WILT' AND 'WITHER-TIP' COMPARED.

The disease described in the present paper differs from 'Wither-Tip' primarily in the fact that in the former the short lateral shoots are affected, in the latter the long terminal shoots; both kinds of shoots bear leaves only and develop from buds produced on long shoots in the previous summer. The difference is not an absolute one, since under certain conditions, particularly if the terminal shoot is injured or checked in growth, the lateral shoots are induced to elongate and are then subject to 'Wither-Tip'.

Although the two have thus much in common, the disease at present under consideration shows certain features that do not appear in typical cases of 'Wither-Tip'. The wilt of the short shoots is noticeable early in the season, i.e. about the time the trees are in bloom, whereas 'Wither-Tip' is not conspicuous until later in the season, when the terminal shoots have reached a length of several inches. In 'Wither-Tip' the disease is confined to the current year's growth, since further extension of the fungus ceases before it reaches the older parts of the twig; the axis of a lateral shoot, on the other hand, is usually so short that the fungal hyphae pass almost directly from the infected leaves to the twig bearing the shoot and a canker of the bark and wood round the insertion of the shoot very frequently results, together with the gummosis of the young xylem elements as described above.

In both forms of disease *Monilia* fructifications may appear on the leaves, under favourable conditions, during spring and summer, but as a rule

they are not found on the bark of the shoots (or on the cankers) until the following winter.¹

VI. THE ECONOMIC IMPORTANCE AND THE CONTROL OF THE DISEASE.

No estimates have yet been obtained as to the losses due to Shoot-Wilt, but the direct damage caused by the disease is probably inappreciable except in certain seasons when there is mild damp weather as the leaf-buds are unfolding. The short shoots are, as already explained, incipient fruit spurs, and the killing of a number of such shoots one year means a corresponding reduction in the number of inflorescences the following year (Fig. 6). The fact that further extension of the mycelium often ceases before the cankers girdle the twigs is of some significance, since such lesions tend to become healed over, so that little direct harm is done in those cases unless many shoots become infected.

Perhaps the chief economic importance of the disease is the fact that not only may the mycelium in the withered leaves give rise to the *Monilia* fructifications during the season in which infection occurs and so cause further dissemination of conidia that year, but the dead shoots and cankers become, in the following season, sources of infection which are easily overlooked. When it is remembered that *Sclerotinia cinerea* infects not only the leaves (as shown in this article) but also the flowers (often causing serious outbreaks of Blossom-Wilt) and the fruit, all possible sources of infection must be taken into consideration if attempts to keep the Brown Rot diseases under control are to be successful.

The cankers on the one-year-old twigs are too small for their excision to be a practical operation, and to cut back behind them would often mean removing a number of incipient fruit spurs. It cannot be over-emphasized that mummied fruit and twigs killed by the Brown Rot fungi should be removed and destroyed by fire whenever this is at all practicable. To supplement this treatment the writer recommends, in cases where the 'Shoot-Wilt' disease is known to be present, the application, in winter, of a caustic alkali wash to which soap has been added, the soap being necessary to ensure a complete wetting of the powdery *Monilia* fructifications. Such a wash has not yet been thoroughly tested as a means of controlling Brown Rot diseases, but it has been found, in experiments on a small scale, that a spray-fluid containing 1 per cent. caustic soda and 1 per cent. soft soap, used as a winter wash shortly before the buds open, will either destroy the fructifications or render them sterile for some weeks.

¹ The writer has found *Monilia* fructifications on the bark of a recently killed plum shoot on one occasion only—on a shoot affected with 'Wither-Tip' in May 1921.

VII. SUMMARY.

1. A wilt of the short shoots of Victoria plum trees is described.
2. The shoots are killed soon after the leaves unfold and mycelium extends from the dead shoots into the twigs bearing them, causing cankers.
3. The disintegration of the infected parts results in gummosis of the tissues, and gum often exudes in drops.
4. A necrosis of the young xylem elements can be traced for several centimetres above and below a canker, but the mycelium extends no farther than the actual canker.
5. The fructifications of *Monilia cinerea* are sometimes to be found on the infected leaves during the summer, but they do not appear on the cankers until the following winter and spring.
6. Conidia taken from a canker in winter had an average size of $11.3 \times 8.4 \mu$, but when the fungus was grown on plums (fruit) in summer the average size of the conidia produced under these conditions was $16.8 \times 12.5 \mu$.
7. Shoot-Wilt has been induced on plum trees by inoculating punctured leaves with conidia of the fungus grown in pure cultures.
8. The fungus causing the disease is *Sclerotinia cinerea*, (Bon.) Schröter, f. *pruni*, as shown by its—
 - (a) morphology,
 - (b) mode of growth in pure cultures,
 - (c) inability to invade the axes of apple inflorescences when flowers are inoculated with conidia,
 - (d) comparatively slow rate of secretion of an oxidase when growing in liquid culture media.

EXPLANATION OF PLATES XIII AND XIV.

PLATE XIII.

- Fig. 1. Typical example of 'Shoot-Wilt'.
- Fig. 2. Two cankers, the result of infection through short shoots, showing a copious flow of gum.
- Figs. 3 and 4. Cankers as seen in the winter following infection of the shoots; at this stage the cankers bear conidial fructification of *Sclerotinia cinerea*.
- Fig. 5. A twig showing the result of infection, through a short shoot.
- Fig. 6. Portion of a plum twig, showing the remains of a short shoot killed during the previous winter, which has now developed into flowering spurs.

second winter after infection; the

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Fig. 8. A twig cut longitudinally to pass through the base of the wilted shoot on the right; there is no definite canker on the twig, but necrosis of the xylem is seen as a dark line on the inner side of the cambium line (natural size).

Fig. 9. Portion of the twig shown in Fig. 8 as seen with a lens ($\times 4$). *c.*, the cambium; *x.*, necrosis of xylem elements shown by an almost continuous line of gum-pockets.

Fig. 10. Section of twig, as seen with a lens ($\times 5$), at 2 cm. above a Shoot-Wilt canker; necrosis of the xylem is seen as an arc of dark spots ('gum-pockets') on the right; condition in June of the same year in which infection occurred.

Fig. 11. Section across a twig at 2 cm. below a Shoot-Wilt canker, showing the 'gum-pockets' embedded in the xylem; condition in March of the year following infection. $\times 5$.

Fig. 12. Section through a canker on a two-year-old twig (about 12 months after infection), showing the callus covering the lesion. $\times 5$.

Fig. 13. Pure culture of *Sclerotinia cinerea*, obtained by placing a particle of infected tissues from a canker on carrot agar, 20 days old, growing at room temperature (slightly reduced). [Compare culture of *S. cinerea* derived from an ascospore, as shown in Ann. Bot., vol. xxxv, Plate VI, Fig. 4.]

Fig. 14. Portion of a twig bearing short shoots, one of which has been killed as a result of inoculating a single leaf (punctured) with conidia of *S. cinerea*.



1



3



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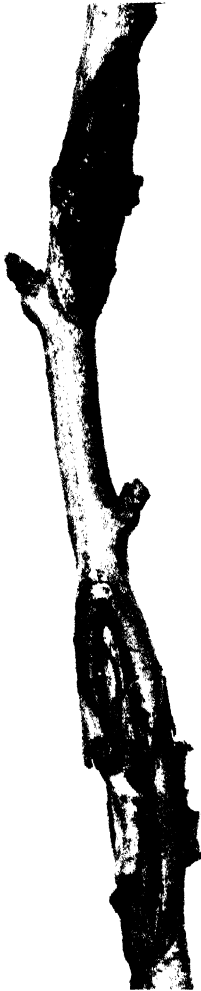


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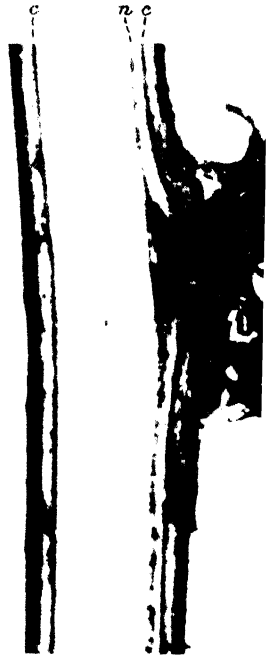
WORMALD-SHOOT-WILT.



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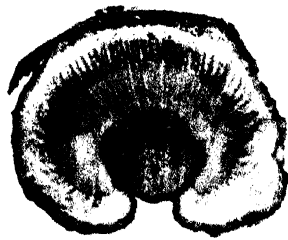
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Herb coll.

The Distribution of Plants in Perthshire in Relation to 'Age and Area'.

BY

J. R. MATTHEWS, M.A., F.L.S.,

Royal Botanic Garden, Edinburgh.

With two Figures in the Text.

A RECENT series of papers from the pen of Dr. Willis has made familiar to students of plant-distribution the theory which its author has styled 'Age and Area'. The principle has been fully worked out by Willis from the study of numerous floras, and the chief contention, that, *on the average*, the older a species is within a given country the greater area it will occupy, is one which is now generally accepted. A review of the hypothesis and of its manifest limitations has been given by Willis (1921).

The applicability of the theory to the flora of Britain may appear doubtful, since the effects of man's occupation are only too well known, the action of man being one of the modifying factors emphasized by Willis. Nevertheless, an analysis of certain portions of the British flora was undertaken by the present writer. The work resolved itself into a cartographic study, since the possibility of examining invasions became the chief point of interest. From this standpoint, a short paper, illustrated by maps, was communicated to Section K at the Edinburgh meeting of the British Association for the Advancement of Science, and abstracts have appeared in the 'Naturalist' (November 1921) and in the 'Journal of Botany' (January 1922).

A recent communication by Willis and Yule (1922) indicates that the distribution of certain *local* floras and certain groups of British animals agrees perfectly with the 'Age and Area' principle. When this paper appeared, I had just completed an examination of the distribution of plants in Perthshire, and since the facts are available they may serve to illustrate how far the hypothesis is applicable to a particularly interesting local flora and, at the same time, emphasize the complexity of the problem in so far as certain constituents of our flora are concerned. I have not sought to deal with size of genera within and without Perthshire. The facts which

I wish to present deal with the range of dispersal in Perthshire as determined by the number of districts occupied.

For botanical purposes Dr. White (1898) divided the county of Perth first into two primary regions, Highland and Lowland, the area of the former being a little more than twice that of the latter. A convenient boundary was found in the line of the 'Great Fault' which runs across the county in a south-westerly direction (Fig. 1). The Lowland area is further subdivided into five, the Highland region into eight districts, the boundaries being largely determined by the chief river systems. Within the Lowland

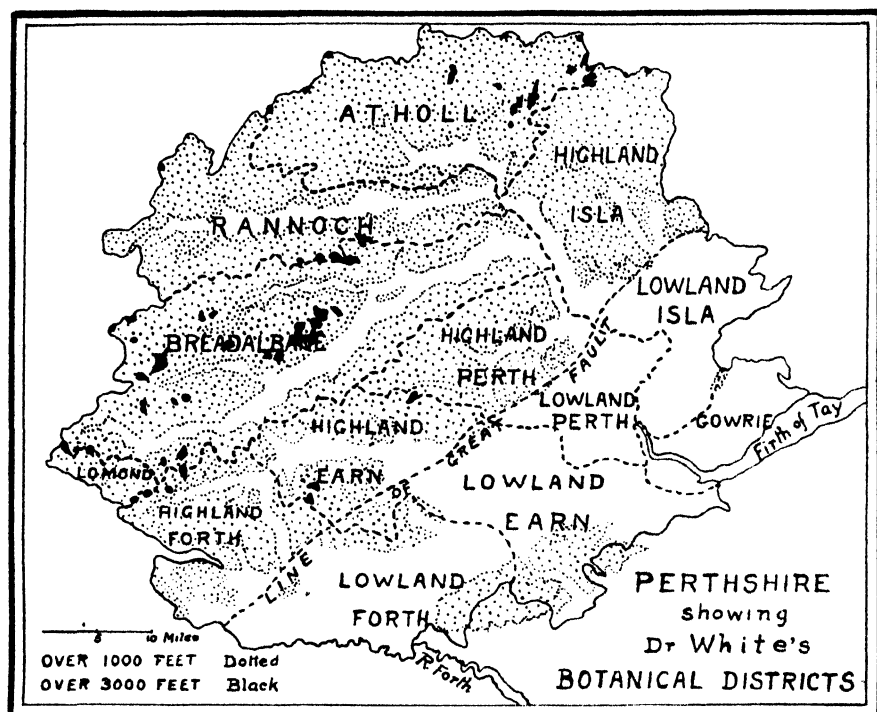


FIG. 1.

region, with an area of 842 square miles, only 91 square miles are over 1,000 ft. The Highland region extends to 1,747 square miles, embracing 1,346 square miles over 1,000 ft., 314 over 2,000 ft., and 17 over 3,000 ft., including 89 peaks which each exceed 3,000 ft., the highest being Ben Lawers (3984 ft.) in Breadalbane. We are thus dealing with a region presenting a very varied topography, with edaphic and epedaphic conditions which differ according to whether the Lowlands or the Highlands are concerned, and whose rivers and mountains present a succession of barriers to the migration of plants from one district to the next. If the application of the 'Age and Area' hypothesis throws any light on the distribution and migration of plants within this region, it may also prove a useful aid

in the search for a solution to the much-debated problem of the origin and distribution of the British flora as a whole.

So far as Perthshire is concerned, the following quotation from White's 'Flora' (1898) gives fair expression to the problem: 'I have been speaking of the British flora and Britain, but with the object of attempting to show that what concerned these in remote ages may have a more modern application to the Perthshire flora and to Perthshire. The various contrivances for the dispersion of plants, to which we have alluded, clearly indicate that the extension of the area of plants is for the benefit of the species. Perthshire is not cut off by the sea from the rest of Britain, therefore the methods by which the British flora reached Britain, when there was a land connexion, may have always been, and still be, operative as regards Perthshire in relation to the rest of Britain provided that climatic conditions are favourable, and that there is room.' It is clear that Dr. White held the view so consistently advocated by Mr. Clement Reid regarding the destruction of Britain's pre-glacial flora during the period of maximum glaciation, and on this view, that the Perthshire flora re-immigrated during post-glacial times, we may, without lengthy discussion on a debated question, proceed to examine the facts of present distribution.

There are 738 flowering plants admitted as 'native species' in the county, and their range of distribution, in terms of the number of districts they occupy, is shown in the following table:

TABLE I.

<i>Occupying.</i>	<i>Number of species in Perthshire.</i>	<i>Percentage.</i>	<i>Same species in Britain.</i>	<i>Percentage.</i>
1. Thirteen districts	190	25.7	258	35.0
2. Twelve "	113	15.3	72	9.8
3. Eleven "	42	5.7	77	10.4
4. Ten "	30	4.1	52	7.1
5. Nine "	34	4.6	58	7.9
6. Eight "	35	4.7	29	3.9
7. Seven "	41	5.6	30	4.1
8. Six "	36	4.9	18	2.4
9. Five "	27	3.7	18	2.4
10. Four "	31	4.2	32	4.3
11. Three "	43	5.8	31	4.2
12. Two "	41	5.6	28	3.8
13. One district	75	10.1	35	4.7

If we mark the rarity of these groups from one to thirteen simply, to avoid awkward fractions, the average rarity for the total number of species is 5.6, while for the same species in Britain, calculated on the same basis from vice-comital distributional data, the rarity is 4.4. Thus, within the limits of Perthshire, species are, on the average, not so widely distributed as they are in Britain. This might be expected from general considerations of geographical position, topographic, climatic, and edaphic factors. But

doubtless, also, the time factor is concerned, since, in general, species will have spread over a great part of Britain before they penetrate far into Perthshire.

Although a large proportion (46·7 per cent.) of the Perthshire flora possesses a wide range, occupying 11–13 districts, a not inconsiderable number of species (159, or 21·5 per cent.) is limited to one, two, or three districts. The numbers, as they stand, do not illustrate that regular descending series, 'from many of wide distribution to few of restricted distribution, which characterizes the 'wides' of Willis's investigations. The figures do not give an entirely satisfactory 'hollow curve pattern'. For we have, in fact, in Perthshire a flora comprising both the common and the rare, and the interesting question is how far this rarity may be due to recent arrival (we are not concerned with endemics) or to other causes.

Now, it is well known that many of the rare species in Perthshire are arctic-alpines, plants of the Highland region essentially. But a larger number of uncommon species belong, in fact, to the Lowland region, while a considerable number overlap, some being predominantly Highland, others chiefly Lowland. Along these lines a further analysis of the flora has been undertaken, the results being set out in Table II.

TABLE II.

<i>Occupying.</i>	<i>Widely distributed.</i>	<i>Chiefly Lowland.</i>	<i>Entirely Lowland.</i>	<i>Chiefly Highland.</i>	<i>Entirely Highland.</i>	<i>Totals.</i>
1. Thirteen districts	190	...	...	...	...	190
2. Twelve "	113	...	...	...	...	113
3. Eleven "	42	...	...	...	...	42
4. Ten "	30	...	...	...	...	30
5. Nine "	27	...	...	7	...	34
6. Eight "	20	...	...	6	9	35
7. Seven "	...	23	...	10	8	41
8. Six "	...	19	...	8	9	36
9. Five "	...	15	7	2	3	27
10. Four "	...	13	11	3	4	31
11. Three "	...	7	18	8	10	43
12. Two "	...	3	27	0	11	41
13. One district	...	0	40	0	35	75
Totals	422	80	103	44	89	738
Rarity	2·2	8·6	11·8	7·7	10·6	5·6
		4·7		9·6		
Rarity in Britain	2·2	5·2	5·9	7·0	11·3	4·4
		3·2		9·9		

In discriminating between common and rarer species it is only the number of districts occupied that is considered, and, for purposes of general discussion, widely distributed species are taken as those occupying eight or more districts. The division of the county into five Lowland and eight Highland districts has rendered necessary an extension of the analysis of the rarer Highland element beyond the limits imposed by the smaller number of Lowland districts.

The 'Age and Area' hypothesis is so well known that it is here unnecessary to do more than point out that at least certain of the results obtained above agree perfectly with the general aspects of the theory. The widely distributed species in Perthshire prove to be common species in Britain. In both the rarity is 2·2. Chiefly Lowland plants show a higher degree of rarity both in Perthshire and in Britain, and these, taken together with the widely-distributed species (i. e. 502 species out of a total of 738), exhibit a remarkably striking descending series, quite comparable with the 'wides' of Willis. But Perthshire possesses 103 rare, entirely Lowland species, as many as 40 of these being limited to a single district. The numbers run in the opposite direction from the 'wides'. Since these species are not endemics, their great rarity cannot be due to recent origin, but, on the view of 'Age and Area', to recent arrival within the county. They are confined very largely to Gowrie and Lowland Earn, where the bulk of the Perthshire flora seems to have entered, and they are thus possibly at an early stage of invasion.

These three groups, widely distributed, chiefly Lowland, and entirely Lowland elements, form the bulk (605 species, or 82 per cent.) of the county flora. They constitute an assemblage of temperate plants and their mass distribution suggests migration from the east, since this temperate flora is concentrated in the Lowlands, as many as 553 species occurring in the small area of Gowrie, while the average number for the five Lowland districts is 518, or 86 per cent. In the Highland region, Highland Isla possesses the largest number, 454 species, but the average number in the Highland districts is 380, or 63 per cent., only. There is thus indicated a pronounced thinning out of the temperate flora towards the interior and mountainous parts of the county, although it is true that many species have spread often far into the Highland valleys. In general, then, this portion of the county flora affords further evidence in favour of 'Age and Area', although the occurrence of an entirely Lowland group, of very marked rarity, may mean that other more vital factors result in certain species lagging behind the steady march of the main body. This is, of course, admitted, and will be due, generally, to ecological barriers, into the details of which in Perthshire we cannot here enter.

Another aspect of the problem arises when the Highland element of the flora is considered. This is largely composed of species which are the recognized arctic-alpine or, at least, boreal members of the British flora. There is no evidence from the facts of present distribution that these plants follow the law of 'Age and Area'. Nor shall we seek to apply it, since if the Highland species have a high degree of rarity, it is not because they are, in general, of recent introduction. We know from geological evidence that arctic-alpine species were formerly more widespread, an arctic flora occurring not only generally throughout Scotland in early post-glacial

times, but extending far south into England during late Pleistocene (Bennie (1896), Lewis (1911), Reid (1911), Chandler (1921)).

It has been estimated that about 13,000 years have elapsed since the beginning of emergence of Scotland and Scandinavia from the ice cap of the last glacial period. Since then an arctic flora has almost disappeared. Numerous species once occurring in Britain are now extinct. Those that remain are confined to our higher hills, and while they may not be visibly

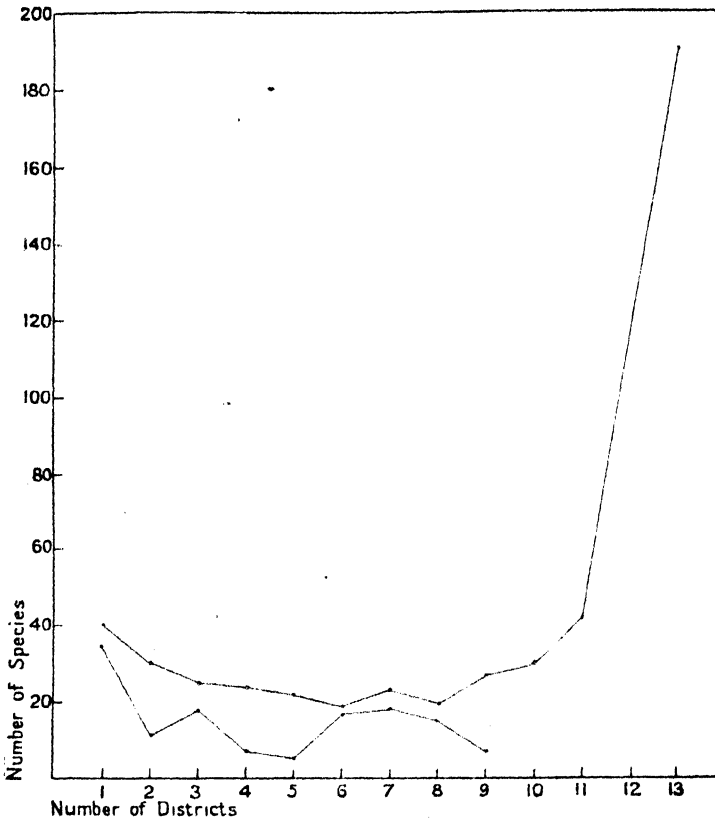


FIG. 2.

disappearing, except from the extravagance of collectors, they must, on a long view, be regarded as relics of an outgoing flora. They are members of that palaearctic flora which once girdled the globe in high latitudes, and which, I believe, will be found to provide a greater number of relics than occurs in a tropical flora. The bulk of the returning, incoming temperate flora, which has replaced the earlier more arctic assemblage, I regard as of comparatively recent date. Much of it is due to and depends upon man's occupation of the country. But as man's migrations and operations must have been determined broadly by the time factor, so would the migration

of those plants which were directly or indirectly affected by human activity. Thus, the mass of the flora, a recent temperate assemblage, fits in, generally speaking, with the ideas expressed in the 'Age and Area' theory. Since the Highland plants are relics, their range will not depend upon age but upon other factors, and their curve of distribution may be, and in Britain is likely to be, irregular. The curve for the species in Perthshire is shown in Fig. 2, and above it is placed the curve for the temperate or general lowland flora. Further comparisons between the outgoing and incoming floras suggest themselves, but these are left for another paper which will deal with the entire Scottish flora.

SUMMARY.

The native flowering plants in Perthshire number 738, of which 605 (or 82 per cent.) belong to a lowland or temperate flora. This element is concentrated in the Lowland districts of the county and thins out in the Highland districts. The distributional data relating to these species, arranged according to number of districts occupied, furnish evidence to show that the general principle expressed in the recent theory of distribution termed 'Age and Area' is applicable to a county flora. Exception to the general bearing of the hypothesis is not unexpectedly found in the distribution of the remaining 133 species, which are essentially arctic-alpine or boreal plants. These, as proved by the fossil record, are relics of a northern flora formerly more widespread, and a gradual elimination since glacial times has produced their present discontinuity of distribution.

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On the Nature of the 'Blade' in certain Monocotyledonous Leaves.

BY

AGNES ARBER, D.Sc., F.L.S.

(Keddey Fletcher-Warr Student of the University of London).

With twenty-nine Figures in the Text.

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I. INTRODUCTION.

IN the course of the last four years I have discussed, in a sequence of papers in this and other journals,¹ the interpretation of the Monocotyledonous leaf in terms of the phyllode theory. In certain cases this interpretation presents little difficulty. There is nothing strained, for instance, in the view that such a leaf as that of *Triglochin maritimum* is a petiolar phyllode; its sheathing base is separated by a ligule from a limb, which is more or less cylindrical in form with a slightly flattened ventral surface—both in its appearance and in its 'radial' anatomy, this limb is distinctly petiolar. But the extension of the phyllode theory to those Monocotyledons which have a leaf-blade showing a general resemblance to the lamina of a Dicotyledon is attended with more difficulty. To many minds there seems, *a priori*, to be an element of improbability in the view, first suggested by Henslow,² that these blades are not true laminae, but are elaborations of the distal region of the petiole. In previous communications I have considered the nature of the leaf-blade in certain special cases, but I propose now to take a wider survey, and to submit the subject to a more rigorous analysis, paying special attention to the comparison between the ontogeny of Monocotyledonous and Dicotyledonous leaf-blades. The observations in the present paper relate to the 'lamina' as it appears in the leaves of examples from among the Helobieae, Principes, Synanthae, Spathiflorae, and Liliiflorae, and also of certain Dicotyledons selected for study because of their apparent resemblance to some of the Monocotyledons in question. In the case of the Glumiflorae I am contenting myself with a passing reference, since I hope to discuss the leaves of the Gramineae and Cyperaceae in a later paper.

I am indebted for material to the Director of the Royal Botanic Gardens, Kew; to the Director and to the Superintendent of the Cambridge Botanic Garden; to Dr. H. E. Durham and to Mr. J. H. Maiden, F.R.S., of Sidney. Two of the illustrations in the present paper are drawn from preparations made by the late Miss Ethel Sargent.

II. OBSERVATIONS ON THE ORIGIN OF THE 'LAMINA' IN THE LEAF OF CERTAIN MONOCOTYLEDONS.

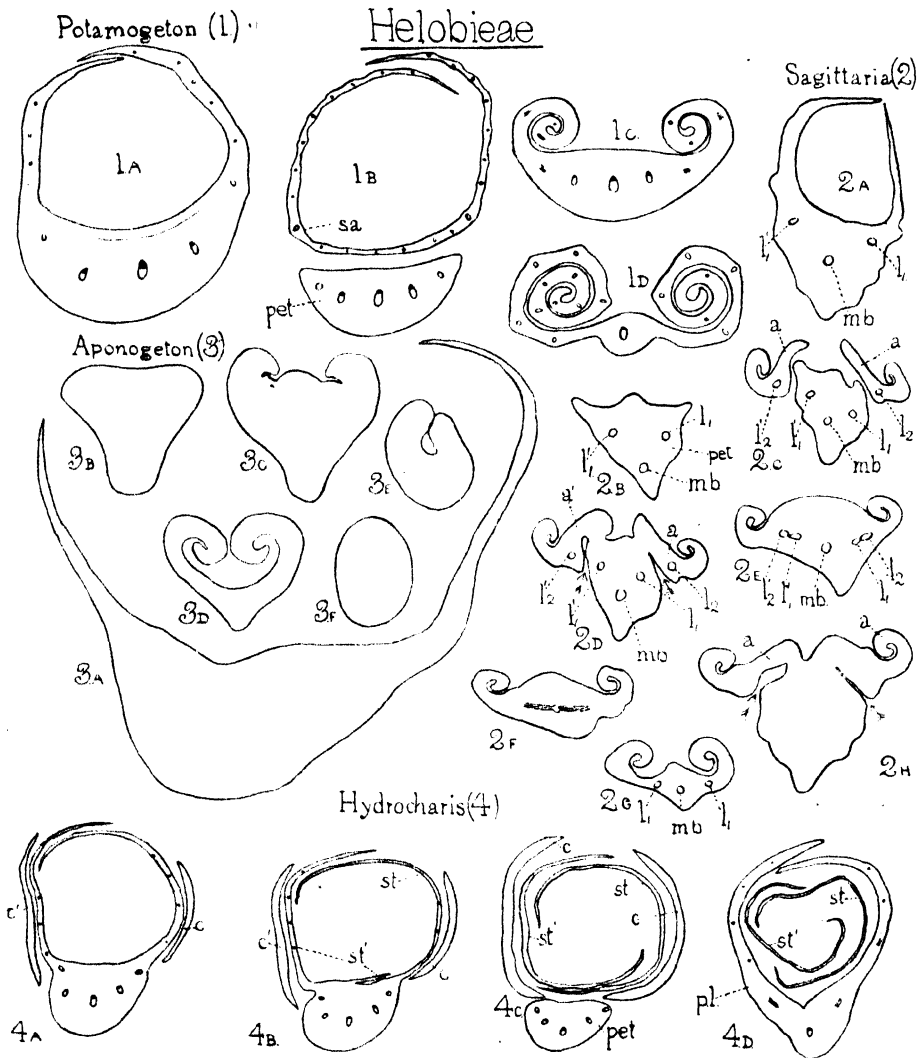
HELOBIEAE—Potamogetonaceae.

Potamogeton.

Sections of the apical buds of shoots of *Potamogeton natans*, L., bearing leaves of the floating type, show that these leaves in their young stages have at their base an extremely short sheathing region. In the case of the leaf illustrated in Figs. 1 A-D, the section represented in Fig. 1 A was the only one, in a series cut to a thickness of 14μ , which could accurately be

¹ Arber, A.: (1918), (1919¹), (1920¹), (1920²), (1920³), (1921¹), (1921²), (1921³), (1922¹), (1922²).

² See reference in Arber, A. (1918), p. 470.



FIGS. 1-4. Figs. 1 A-D, *Potamogeton natans*, L., series of transverse sections from below upwards through a young leaf, from sheath, Fig. 1 A, to 'lamina', Fig. 1 D ($\times 47$); *pet.*, petiole; *s.a.*, stipula adnata. Fig. 2, *Sagittaria sagittifolia*, L., Figs. 2 A-G, serial sections through one young leaf ($\times 23$) from sheath, Fig. 2 A, to 'lamina', Fig. 2 G; *a.* and *a'*, auricles; *pet.*, petiole; *m.b.*, median bundle; *l.*₁ and *l'*₁, main lateral bundles; *l.*₂ and *l'*₂, bundles given off from *l.*₁ and *l'*₁ to supply auricles; Fig. 2 H, transverse section of another young leaf ($\times 23$) at level of detachment of auricles, vascular bundles omitted. In Figs. 2 D and 2 H the arrows indicate the invaginations which detach the auricles. Figs. 3 A-F, *Aponogeton distachyum*, Thunb., series of transverse sections through one leaf from below upwards ($\times 47$), vascular bundles omitted. Fig. 4 A-D, *Hydrocharis morsus-ranae*, L., series of transverse sections through one leaf passing from sheath, Fig. 4 A, to 'lamina', *pl.*, Fig. 4 D; *pet.*, petiole; *st.* and *st'*, stipules; *c.* and *c'*, basal cordate lobes of 'lamina' ($\times 23$).

described as passing through the leaf-sheath; the section next below showed fusion between leaf and axis, while that next above revealed an early stage in the detachment of the *stipula adnata*—a detachment whose plane is already indicated in Fig. 1 A. The section drawn in Fig. 1 B passes through the petiole (*pet.*) and shows the stipule (*s.a.*) as a free, sheathing structure, open on the side remote from the petiole. Fig. 1 C shows the initiation of the 'lamina' by the development of wing-like marginal outgrowths, which become coiled as they elongate. In Fig. 1 D the definitive form of the limb is reached. For comparison, I have cut serial sections through the apical buds of a second species of *Potamogeton* (unidentified); it was of a somewhat different vegetative type from *P. natans*, as it had broad submerged leaves, but I found that its leaf development proceeded on essentially the same lines as in that species.

HELOBIEAE—Aponogetonaceae.

Aponogeton.

The developing leaves of the apical bud of *Aponogeton distachyum*, Thunb., have at the base a conspicuous sheathing region (Fig. 3 A), which is succeeded by a petiole, more or less triangular in section (Fig. 3 B). The 'lamina' is formed by outgrowths from the lateral margins of the ventral surface of the petiole (Fig. 3 C), which become coiled as they elongate (Fig. 3 D). Towards the tip, the blade, as is so often the case in Monocotyledonous leaves, loses its dorsiventral character, and at the extreme distal end it enters on a solid cylindrical phase (Figs. 3 E and F).¹

HELOBIEAE—Alismaceae.

Sagittaria.

In the case of *Sagittaria sagittifolia*, L., I have studied serial sections through buds consisting of leaves of the mature 'arrow-head' type. In Figs. 2 A-C, drawn from a single leaf belonging to such a bud, only the three main vascular strands are indicated—*m.b.*, the median bundle, and *l.*₁ and *l'*₁, the main laterals. Fig. 2 A shows the sheathing structure at the leaf-base, which passes up into a triangular petiole (Fig. 2 B). Fig. 2 C is from a section traversing the distal region of the stalk; the tips of the auricles (*a.* and *a'*) with their bundles (*l.*₂ and *l'*₂) are cut on either side. Fig. 2 D shows the connexion of the auricles with the petiole. In Fig. 2 E the origin of the vascular supply of the auricles can be traced; the lateral bundles (*l.*₁ and *l'*₁) bifurcate—the outer half in each case being destined to pass down into an auricle. This section also indicates how the main part of the 'blade' comes into existence by the development of lateral wings from the petiole. At a slightly higher level there is a fusion between the median bundle and the laterals (Fig. 2 F); the general arrangement of the developing veins is perhaps not inconsistent with the morphological

¹ Arber, A. (1922²).

interpretation of the arrow-head 'lamina' which I have suggested in a former paper.¹ In Fig. 2 G the upper part of the leaf-limb is reached. Fig. 2 D shows the detachment of the auricles by means of invaginations, indicated by arrows, and the same stage is also seen in Fig. 2 H, drawn from another leaf.

HELOBIEAE—Hydrocharitaceae.

Hydrocharis.

Microtome sections through the leaf-buds of *Hydrocharis Morsusranae*, L., show that the orbicular floating leaves are sheathing at the base (Fig. 4 A). The cordate basal lobes of the 'lamina' are cut through on either side and hence appear as detached objects (*c.* and *c'*). The sheath passes upwards into the paired stipules (*st.* and *st'*), which are seen in Fig. 4 B in process of separation from the petiole. In Fig. 4 C the two stipules and the petiole (*pet.*) are entirely free from one another. Fig. 4 D shows the 'lamina' (*l.*), which is produced by lateral expansion of the petiole.

PRINCIPES—Palmae.

I have considered the Palm-leaf in some detail in a previous paper,² and have shown that in this family the apparently plicate leaf-limb originates, not by folding—as has been generally supposed—but by means of a series of invaginations involving the petiolar tissues. So I shall now refer very briefly to two cases only.

Areca.

Figs. 5 A-E illustrate the passage from the sheath (Fig. 5 A) through the petiole (Fig. 5 B) to the 'lamina' (Fig. 5 E) in *Areca sapida*, Soland. It will be seen that the plication arises by means of a series of dorsal and ventral invaginations penetrating between the main vascular strands (*m.b.*, *l.*₁, *l.*₂, *l.*₃, *l'*₁, *l'*₂, *l'*₃), which can be followed from section to section. The process is a rapid one; a distance of only 0.35 mm. intervenes between the first sign of the first invagination and the stage represented in Fig. 5 D.

Orcodoxa.

I add three drawings (Figs. 6 A-C) to illustrate the relations, in form and skeletal system, between the petiole and 'lamina' in a second case—*Orcodoxa regia*, H. B. et K. The bundles are lettered as in Fig. 5. The first pair of invaginations, indicated by arrows, come into view in Fig. 6 B.

SYNANTHAE—Cyclanthaceae.

Carludovica.

Many years ago Eichler,³ in his memoir on the developmental history of Palm-leaves, drew attention to their resemblance to those of the Cyclanthaceae—a comparison which has frequently been emphasized by

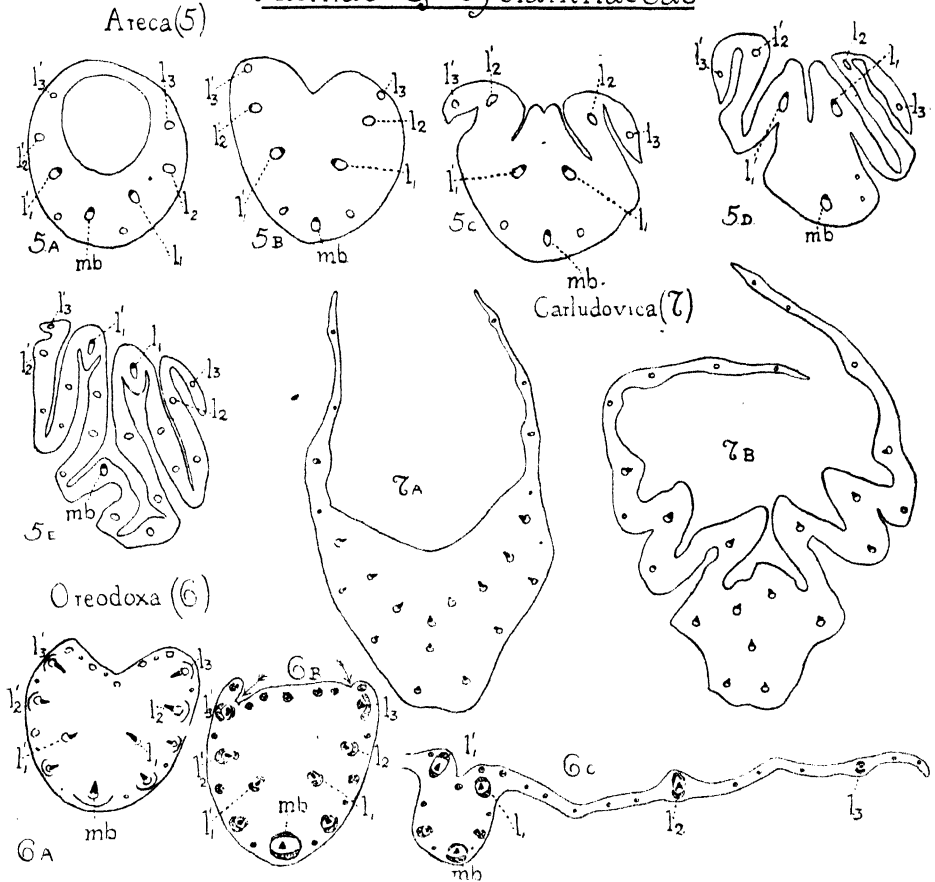
¹ Arber, A. (1921²).

² Arber, A. (1922¹).

³ Eichler, A. W. (1885).

more recent writers.¹ Eichler interprets the leaves of the Cyclanthaceae as owing their form to folding, the agency to which he also attributes the plicated appearance of the Palm-leaf. I have found, however, on studying

Palmae & Cyclanthaceae



FIGS. 5-7. Figs. 5 A-E, *Areca sapida*, Soland., series of transverse sections ($\times 47$) through one leaf, from sheath, Fig. 5 A, to plicate 'lamina', Fig. 5 E; *m.b.*, median bundle; *l*₁, *l*₂, *l*₃, *l'*₁, *l'*₂, *l'*₃, principal lateral bundles. In Figs. 5 C-E the invaginations are shown which penetrate between the bundles of the petiole, Fig. 5 B. Figs. 6 A-E, *Oreodoxa regia*, H. B. et K., sections from a series through the first foliage leaf (third plumular leaf) of a seedling ($\times 14$). Lettering of bundles as in Fig. 5; Fig. 6 A, petiole; Fig. 6 B, first signs of invaginations indicated by arrows; Fig. 6 C, half the 'lamina', showing eventual distribution of bundles. Figs. 7 A, B, *Carludovica Plumerii*, Kunth, transverse sections of young leaf passing through sheath, Fig. 7 A, and regions where invagination begins, Fig. 7 B ($\times 14$).

the leaves of *Carludovica*, that here, as in the Palms, the process by which the 'lamina' arises is rather to be described as *invagination* of the petiolar tissues. In a young leaf of *Carludovica Plumerii*, Kunth, which I examined, there was no sharp distinction between sheath (Fig. 7 A) and petiole, but

¹ Hirmer, M. (1919).

a series of dorsal and ventral invaginations made their appearance in the sheathing region, and, penetrating between the bundles, produced a 'plicate' form (Fig. 7 B). In the case of *C. rotundifolia*, H. Wendl., I was only able to obtain a much older leaf; in this leaf there was a well-marked cylindrical petiole, in which the process of invagination took place.

SPATHIFLORAE—Araceae.

Calla.

The leaves of *Calla palustris*, L., have a sheathing base enclosing the younger leaves (Fig. 8 A), continued upwards, on the ventral side, into a conspicuous *stipula adnata* (s.a.) (Fig. 8 B). When this stipule becomes free (Fig. 8 C), the petiole (*pet.*) assumes its definitive form. In Fig. 8 D we have reached a point above the top of the *stipula adnata*, and the two basal lobes of the cordate 'lamina' (*c.* and *c'*.) are cut across at a level below their connexion with the petiole. Fig. 8 E shows the detachment of these lobes by a pair of invaginations. In Fig. 8 F we see the 'lamina' arising by lateral winging of the petiole, while in Fig. 8 G it is completely formed. Figs. 8 H and I show its termination in a solid apex¹ in which all dorsiventrality is lost, and in which the bundles fuse into a vascular plexus. The solid tip of a younger leaf (*f.*₂) is shown in Fig. 8 C.

Arum.

The leaf-development in *Arum italicum*, Mill. (Figs. 9 A-F) closely recalls that just described for *Calla*. The auricles (*a.* and *a'*., Fig. 9 C) resemble the cordate lobes at the base of the 'blade' in *Calla*, and they are detached by means of similar invaginations (Fig. 9 D). The bundles which pass into the auricles are at this stage scarcely differentiated, and I have not been able to trace their origin with certainty. In Fig. 9 B the top of the sheath (*s.*) takes a form which recalls the *stipula adnata* of *Calla*, but this is perhaps an exceptional development, for in serial sections of two other young leaves, the sheath was found to pass uniformly into the petiole, without this ligulate appearance at the upper margin.

Figs. 10 A-E represent the passage from sheath to apex in the first plumular leaf of *Arum maculatum*, L. This leaf is of the simple, non-auricled type, and there is a suggestion, in the transition from the petiole (Fig. 10 C) to the 'lamina' (Fig. 10 D), that invagination may play some part in the development of the latter.

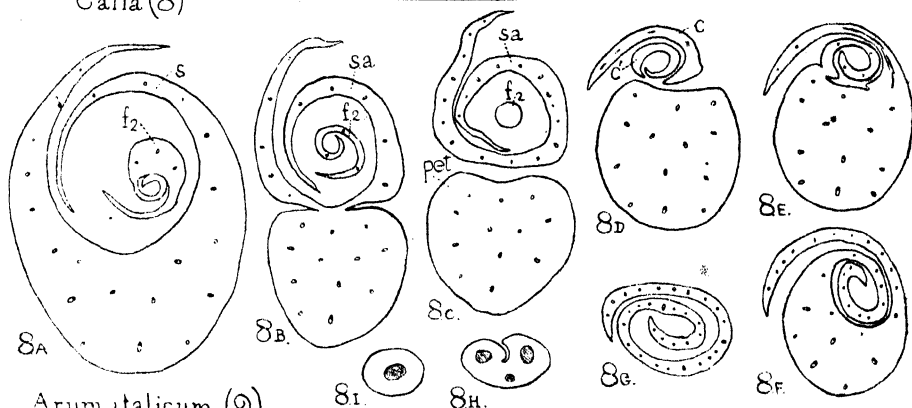
Epipremnum.

For comparison with *Calla* and *Arum*, I cut sections through a lateral bud of *Epipremnum mirabile*, Schott, and I found that the leaf in its early stages (Figs. 11 A-D) conforms to the same type as in the two former genera. The detachment of the auricles (*a.* and *a'*., Figs. 11 A and B) and

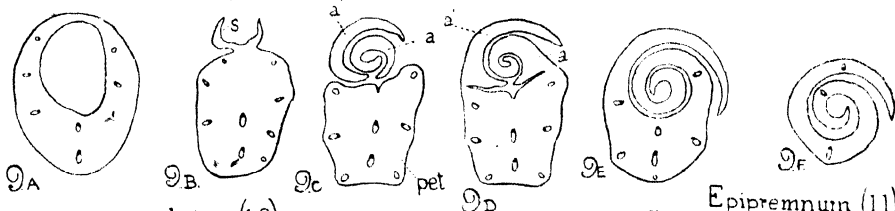
¹ Arber, A. (1922²).

Araceae

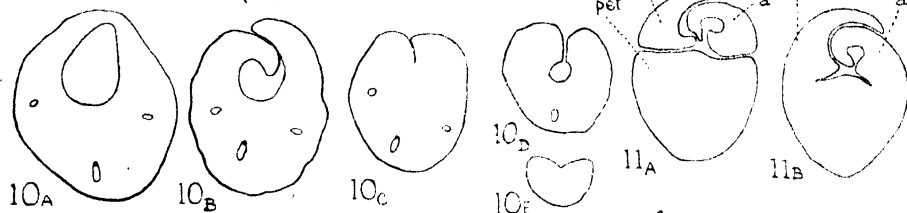
Calla (8)



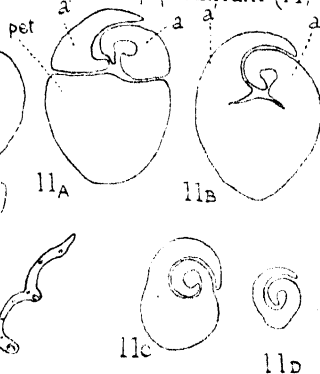
Arum italicum (9)



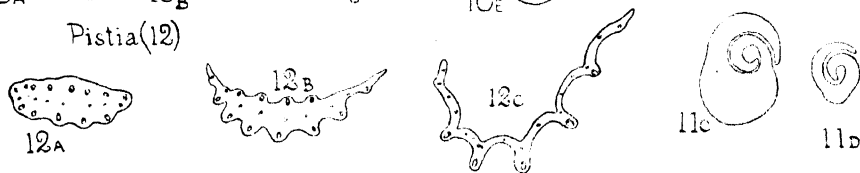
Arum maculatum (10)



Epipremnum (11)



Pistia (12)



FIGS. 8-12. Figs. 8 A-I, *Calla palustris*, L., series of transverse sections through a young leaf ($\times 23$); Fig. 8 A, sheath enclosing upper part of sheath of next leaf, f_2 ; Fig. 8 B, *stipula adnata*, s.a., just becoming detached; the lamina of f_2 is shown; Fig. 8 C, *stipula adnata*, s.a., free from petiole. *pet.*: solid apex of the second leaf, f_2 , is cut through; Fig. 8 D, cordate lobes, $c.$ and c' , of base of 'lamina' are seen on the adaxial side of the petiole, *pet.*; Fig. 8 E, cordate lobes being detached by invagination; Figs. 8 F and G, 'lamina'; Figs. 8 H and I, solid apex of leaf, in which vascular bundles unite to a plexus (shaded). Figs. 9 A-F, *Arum italicum*, Mill., series of transverse sections through one leaf ($\times 23$); Fig. 9 B passes through the extreme upper limit of the sheath, $s.$; Fig. 9 D shows the detachment of the auricles, $a.$ and a' , by means of invaginations. (Figs. 9 A-F slightly reconstructed as the sections were broken.) Figs. 10 A-E, *Arum maculatum*, L., series of transverse sections through first plumular leaf of seedling, from preparations in Miss Sargent's collection ($\times 47$); Figs. 10 A and 10 B, sheath; Fig. 10 C, petiole; Fig. 10 D, 'lamina'; Fig. 10 E, solid apex. Figs. 11 A-D, *Epipremnum mirabile*, Schott, series of transverse sections through a single leaf, sheath and vascular bundles omitted ($\times 47$); Fig. 11 A, just below level of attachment of auricles, $a.$ and a' , to petiole; Fig. 11 B, level at which auricles are connected with petiole; Figs. 11 C and D, 'lamina'. (Fig. 11 A, slightly reconstructed as section broken.) Figs. 12 A-C, *Pistia Stratiotes*, L., series of transverse sections through one young leaf ($\times 14$), leaf-sheath, ligule, hairs, and lacunae, omitted; Fig. 12 A, narrow basal region; Fig. 12 C, broader distal region.

the development of the 'lamina' (Figs. 11 C and D) take place in identically the same way.

Pistia.

The leaf of *Pistia Stratiotes*, L., differs markedly from that of the three genera of Araceae already considered, both in its mature form and in its ontogeny. As I have described the leaf in a former paper,¹ and brought forward anatomical evidence for regarding it as a petiolar phyllode, I will here confine myself to the question of the origin of the thin, lamina-like, distal region of the limb. The basal part of the limb is shown in Fig. 12 A; it is solid and petiole-like. From Figs. 12 B and C it will be recognized that the development of the limb is due to a series of major dorsal invaginations and minor ventral invaginations, located between the main bundles, associated with lateral expansion of the tissue connecting the bundles.

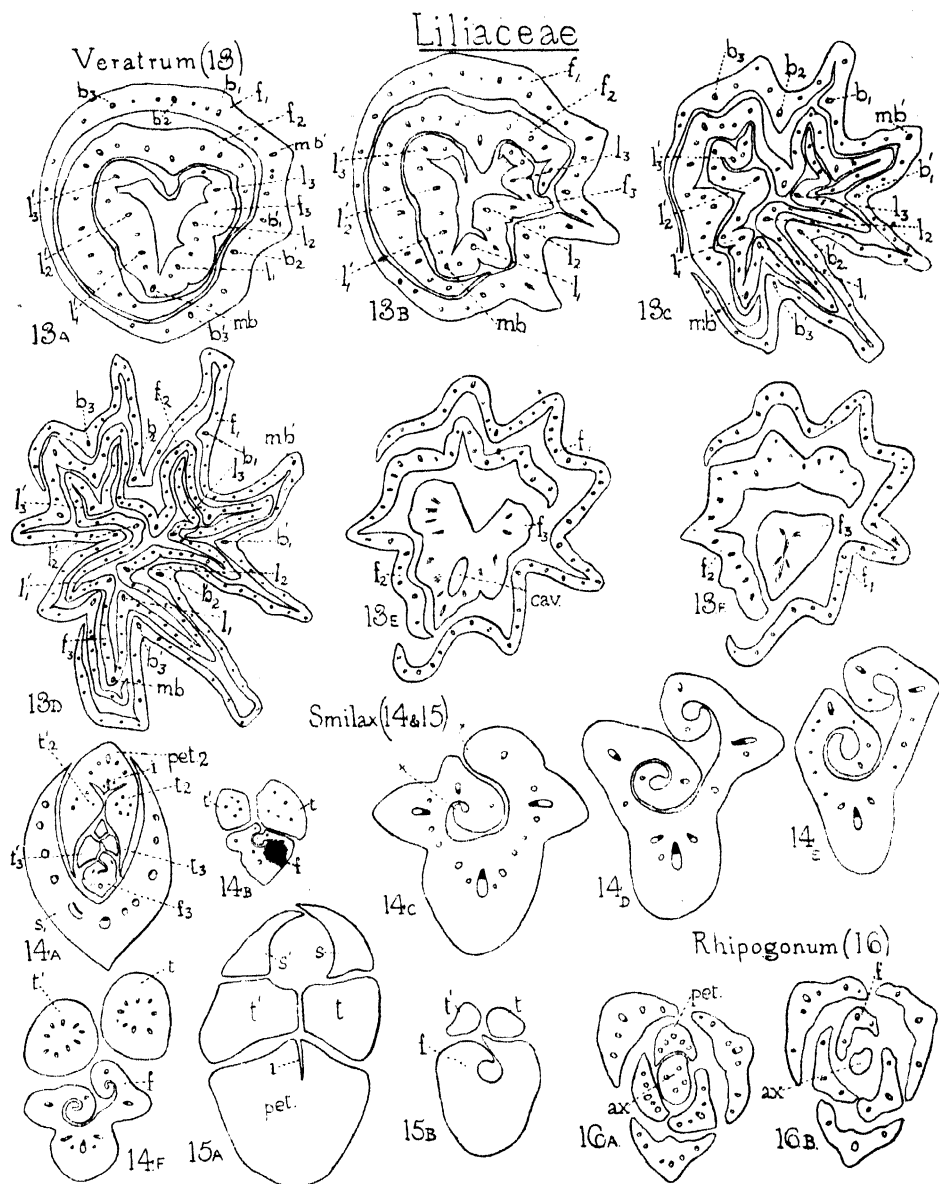
LILIIFLORAE—Liliaceae.

Veratrum.

In a previous paper² I have studied the leaves of *Veratrum album*, L., in connexion with their prolonged cambial activity. The abbreviated axis bears annually a number of leaves, each with a cylindrical elongated leaf-sheath, succeeded by an ovate blade, which is plicated in its young stages, and retains a channelled appearance throughout life. In order to follow the origin and development of the 'blade', I cut serial transverse sections through the terminal underground leaf-bud of a plant cultivated under the name of *Veratrum 'album nigrum'*. This bud included a large number of leaves, but, for simplicity, I have only illustrated in Figs. 13 A-F the development of three of the younger leaves, which we may distinguish as f_1 , f_2 , and f_3 . Fig. 13 A, drawn from a section cut a little above the extreme base of the bud, showed the closed sheaths of the three leaves in question. We may choose the youngest leaf, f_3 , in which to follow the development of the 'lamina'. In Fig. 13 A, although the limit of the closed sheath is not reached, and still more distinctly in Figs. 13 B and C, in which the passage to the blade is taking place, we observe that the leaf is losing its simplicity of form and is becoming deeply channelled by a series of invaginations (the more pronounced appearance of the invaginations on the right-hand side of the diagrams is merely the result of a slight obliquity in this series of sections). The positions of the grooves in the individual leaves is not haphazard, but follows a regular and definite plan. It will be recognized from Fig. 13 C that there is a dorsal invagination opposite each of the six main lateral vascular strands (l_1 , l_2 , l_3 , l'_1 , l'_2 , l'_3) and a ventral invagination between l_3 and l_2 , l_2 and l_1 , l'_3 and l'_2 , l'_2 and l'_1 , and also one between l_1 and l'_1 , i.e. opposite *m.b.* The result is that

¹ Arber, A. (1919¹).

² Arber, A. (1919²).



FIGS. 13-16. Figs. 13 A-F, *Veratrum 'album nigrum'*, sections from a slightly oblique transverse series through a shoot apex, the three innermost (f_1 , f_2 , and f_3) of the numerous leaves being alone represented ($\times 14$). Bundles of f_3 lettered mb (median bundle) and l_1 , l_2 , l_3 , l'_1 , l'_2 , l'_3 (lateral bundles). In Figs. 13 A, C, and D, the bundles of f_1 are also lettered mb (median bundle), b_1 , b_2 , b_3 , b'_1 , b'_2 , b'_3 (lateral bundles). Series ranges from Fig. 13 A, sheathing region of the three leaves, to Fig. 13 F, which passes through the solid apex of f_3 , and near the apex of f_1 and f_2 ; in Fig. 13 E there is a cavity ($cav.$) in f_1 , showing that the apex is hooded. Figs. 14 A-F, *Smilax herbacea*, L., Fig. 14 A, transverse section of apical bud ($\times 14$); the sheathing base of leaf s_1 encloses a second leaf, whose petiole, pet_2 , showing ventral invagination, i , is cut at the level of attachment of the tendrils, t_2 and t'_2 ; a third leaf is cut through the 'lamina', f_3 , and the two free tendrils, t_3 and t'_3 ; Fig. 14 B, transverse section of another leaf passing through the lamina, f_1 , at more

the median bundle comes to occupy the base of a channel running the length of the blade, while the main laterals, L_1 and L'_1 , occupy the ridges which on either side bound the median channel, and the laterals, L_2 and L'_2 , L_3 and L'_3 , are located in the ridges separating two pairs of channels which run on either side of the median channel and parallel to it. In Figs. 13 A, C, and D I have labelled the vascular bundles in f_1 as well as in f_3 , in order to demonstrate that development proceeds on the same lines in both cases. In Figs. 13 C and D the median bundle of f_1 ($m.b'$) is seen, as in f_3 , at the base of the groove between the two main laterals, b_1 and b'_1 , while b_1 , b_2 , b_3 , b'_1 , b'_2 , b'_3 , occupy ridges separated by invaginations. That b_1 is not placed at the actual summit of a ridge, is an accident due to the pressure of the more external leaves which have been omitted in the drawing. It will be noted in all three leaves that the deepening of the invaginations is associated with a thinning of the leaf-substance; this can be seen with great distinctness on comparing, for instance, the segment of the leaf f_1 between the bundles b'_3 and b'_2 , in Figs. 13 A and D. The two bundles remain practically as fixed points, but whereas in Fig. 13 A they are connected by a simple segment of relatively thick leaf-tissue, in Fig. 13 D they have between them a deep fold, whose substance is attenuated to about one-third the thickness of the corresponding uninvginated region in Fig. 13 A.

In Figs. 13 E and F we witness the changes which the blade undergoes in passing towards the apex. In the case of f_3 , all the ventral invaginations, except the median one, have died out, while the dorsal grooves are now reduced to inconspicuous indentations. The apex is slightly hooded, so that a cavity (*cav.*) occurs in the transverse section at this level. The apex thus corresponds to that of the plicate first leaf of the Palm, *Pritchardia filifera*, Lind., which I have described elsewhere.¹ In Fig. 10 F the apex is solid and all the invaginations have disappeared, while the bundles, which were oblique in Fig. 13 E, are now running together, meeting one another almost horizontally.

Smilax.

In a previous paper² I have considered the general morphology of the *Smilax* leaf, without, however, discussing the origin of the 'blade', the question with which we are here concerned. Serial sections through the shoot apex of *S. herbacea*, L., show that at a very early stage—in fact even before

advanced stage, and tendrils, t , and t' . ($\times 14$); Figs. 14 C-E, series of sections through developing 'lamina' of another leaf ($\times 23$); the points marked with a cross in Fig. 14 C will eventually become the margins of the 'lamina'; Fig. 14 F, section of another leaf ($\times 14$) to show developing lamina, f , and tendrils, t , and t' . Figs. 15 A and B, *Smilax laurifolia*, L., two sections of one young leaf ($\times 47$); Fig. 15 A passes through leaf at level just above sheath and shows wings of sheath, s , and s' , tendrils t , and t' , and petiole, pet , penetrated by ventral invagination, i ; Fig. 15 B, same leaf at higher level, just below tips of tendrils; f , lamina. Figs. 16 A and B, *Rhipogonum album*, R.Br., two sections from transverse series through apical bud ($\times 23$); ax , axis; the petiole, pet , of youngest leaf is just detached in Fig. 16 A, and the same leaf is represented by the lamina, f , in Fig. 16 B.

¹ Arber, A. (1922¹), Fig. 5 E, p. 256.

² Arber, A. (1920²).

the tendrils separate from the petiole—the petiolar tissue begins to be penetrated by a single invagination (*i.*) passing from the ventral surface between two of the vascular bundles. This stage is seen in Fig. 14 A in the case of the petiole marked *pet.*₂, to which the tendrils *t.*₂ and *t'*₂ are still attached. A little higher, where the tendrils have separated from the petiole, this invagination is found to have passed more deeply into the tissues, following a curved path, so that the future form of the 'lamina' is already indicated (*f.*₃ in Fig. 14 A and *f.* in Fig. 14 B). In older leaves (Figs. 14 C–F) we can follow the further development of the invagination, which—in association with a wing-like outgrowth of the two newly formed leaf margins, marked with crosses in Fig. 14 C—is responsible for the ultimate shape of the 'lamina'.

Rhipogonum.

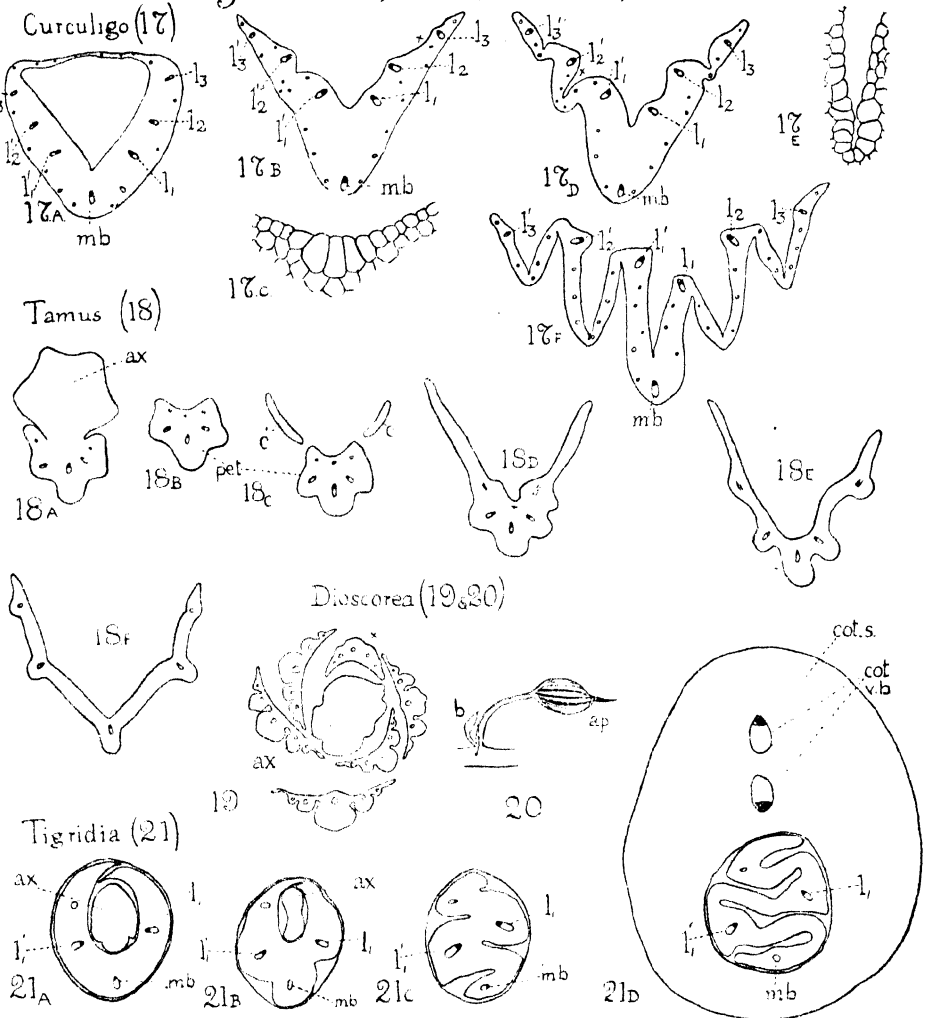
By the kindness of Mr. J. H. Maiden, F.R.S., I have been able to examine young shoots of *Rhipogonum album*, R. Br., from the Botanic Gardens, Sidney, N.S.W. Microtome sections, through the stem apex bearing young leaves, show that in this plant the leaves have no broad basal sheath, but the petiole seems to be attached directly to the axis. This petiole passes into the 'lamina' by a gradual process—becoming broader and thinner the farther it departs from its level of attachment to the stem (cf. *pet.* in Fig. 16 A, with *f.* in Fig. 16 B). It will be recognized, on comparing Fig. 16 with Figs. 14 and 15, that both in the general ontogeny of the leaf and in the origin of the 'lamina', there are much wider divergences between *Smilax* and *Rhipogonum* than one might naturally expect, considering that the latter genus—with *Heterosmilax*—is assigned to the tribe Smilacoideae. The differences are indeed of so essential a nature as to suggest a doubt as to the close affinity assumed to exist between *Smilax* and *Rhipogonum*—a doubt which is not set at rest by a consideration of the reproductive shoots of the two genera, for *Rhipogonum* differs from *Smilax* not only in its hermaphrodite flowers, but also in the characters of the inflorescence.

LILIIFLORAE—Amaryllidaceae.

Curculigo.

Curculigo is one of the few Monocotyledons, outside the Palms and Cyclanthaceae, which have a broad plicate leaf-limb. Just as in the case of these groups, the plicate appearance of the blade of *Curculigo* has hitherto been attributed to folding.¹ But, as Figs. 17 B, D, and F show, the ultimate leaf-form in *C. recurvata*, Dryand., is due, in reality, to a series of alternating dorsal and ventral invaginations of the petiole (or sheath). The 'lamina' development strikingly recalls that of *Veratrum*, already described (pp. 337–9); we find here precisely the same arrangement of a ventral sinus opposite the median bundle, *m.b.*, and four other parallel ventral

¹ Hirmer, M. (1919).

Amaryllidaceae, Dioscoreaceae, Iridaceae

FIGS. 17-21. Figs. 17 A-F, *Curculigo recurvata*, Dryand. Figs. 17 A, B, D, F, series of transverse sections through one leaf, passing from sheath, Fig. 17 A, to 'plicate lamina', Fig. 17 F ($\times 14$); m.b., median bundle; $l_1, l_2, l_3, l'_1, l'_2, l'_3$, main laterals: Fig. 17 C, epidermis at region marked with cross in Fig. 17 B ($\times 77$); Fig. 17 E, epidermis of base of groove marked with cross in Fig. 17 D ($\times 77$). Figs. 18 A-F, *Tamus communis*, L., series of transverse sections through one leaf ($\times 23$) passing from attachment of leaf and axis (ax., Fig. 18 A) to 'lamina' (Fig. 18 F); c. and c', cordate basal lobes of 'lamina' cut on either side of upper part of petiole, pet. Fig. 19, *Dioscorea sativa*, L., transverse section of apical bud, passing through axis, ax., and a series of leaves of which the one marked with a cross is cut nearest to its level of attachment ($\times 14$). Fig. 20, *Dioscorea* sp., young leaf with axillary bud, b, to show narrow pointed apex, ap., which is becoming brown and shrivelled (about nat. size). Figs. 21 A-D, *Tigridia Pringlei*, S. Wats., sections through first plumular leaf, from sheath, Fig. 21 A, to plicate 'lamina', Fig. 21 D, from a series in Miss Sargent's collection ($\times 47$). The outer line represents the inner epidermis of the cotyledon sheath (cot. s.), which is only shown completely in Fig. 21 D; ax., plumular bud; m.b., l_1 and l'_1 , main bundles of first plumular leaf; cot. v. b., vascular bundle of cotyledon, which is cut twice, as it doubles on itself.

sinuses between the main lateral bundles, l_1 and l_2 , l_2 and l_3 , l'_1 and l'_2 , l'_2 and l'_3 . In addition there are, as in *Veratrum*, dorsal sinuses opposite l_1 , l_2 , l'_1 , and l'_2 . The result is that the final arrangement of the main bundles in relation to the ridges and grooves exactly follows the scheme outlined in the case of *Veratrum*.

It is interesting to notice that in *Curculigo recurvata* the first sign that invagination is about to take place is given by a local enlargement of the epidermal cells. This hypertrophy begins before the leaf shows any change of form (Fig. 17 C), and in later stages the enlarged epidermal cells can still be recognized at the base of the grooves (Fig. 17 E).

LILIIFLORAE—Dioscoreaceae.

Tamus.

The leaf of *Tamus communis*, L., is peculiar in the complete absence of any basal region of a sheathing character. Fig. 18 A shows the petiole in the act of becoming detached from the axis (*ax.*); in Fig. 18 B it is free, while in Fig. 18 C the basal lobes (*c.* and *c'.*) of the cordate 'lamina' are seen on either side. Fig. 18 D marks the junction of stalk and blade, while Figs. 18 E and F show how this 'blade' is produced by marginal winging of the petiole and separation of the main bundles. The ribbing of the lower surface of the leaf is brought about by extremely slight dorsal invaginations between the bundles.

Dioscorea.

The ontogeny of the leaf in the case of *Dioscorea sativa*, L., and *D. divaricata*, Blanco, is similar to that of *Tamus communis*, but it will be recognized from Fig. 19 that invagination plays a much more conspicuous part than in the latter genus—giving rise to the deep grooving of the under surface of the young leaf; in this sketch, the section with almost entire outline (marked with a cross) is that of the leaf which is nearest to its point of attachment, while the other leaves, which are cut at higher levels, show deeper grooving.

A noticeable feature of the leaves of the Yams is the differentiated apical region (*ap.* in Fig. 20), which in the young leaf looks almost like a separate lamina. Sir David Prain, F.R.S., was so kind as to draw my attention some years ago to this curious structure; in order to try and understand it, I have studied serial sections through leaf-buds, and I have come to the conclusion that the distal region of the *Dioscorea* leaf has no special morphological significance. It cannot, I think, be treated as a case apart; it belongs to that class of leaf-apex which Raciborski¹ named 'forerunner tips', and which he showed to be associated with the climbing habit, not only in the Dioscoreaceae and in *Smilax*, but also in various Dicotyledons. I find that the leaf-tips of the Yams do not differ from the

¹ Raciborski, M. (1900).

remainder of the limb, except in the fact that they come to maturity precociously, while the rest of the leaf is still tiny and embryonic. Their early development seems to me a phenomenon of the same order as the very early development of the tendrils in the case of *Smilax*—a peculiarity also associated with the climbing habit. The forerunner tip is the most conspicuous feature of the Yam-leaf while it is quite young, but the rest of the limb soon catches it up, and then outstrips it, so that finally it sinks into entire insignificance. But such a reversal of the order of things is not a unique occurrence; it is, indeed, by no means unusual for the proportion of parts to alter completely in the course of ontogeny. In the embryonic leaf of *Narcissus* sp., for example, the small limb forms a mere appendage of the conspicuous sheath, but at maturity the relations are reversed, and the limb is the only obvious region, while the sheath, which originally exceeded it in size, now occupies only a minute proportion of the length of the leaf.¹

LILIFLORAE—Iridaceae.

Since I have already dealt in this journal with the leaf-structure of the various tribes of the Iridaceae,² I will confine myself now to a single illustrative case.

Tigridia.

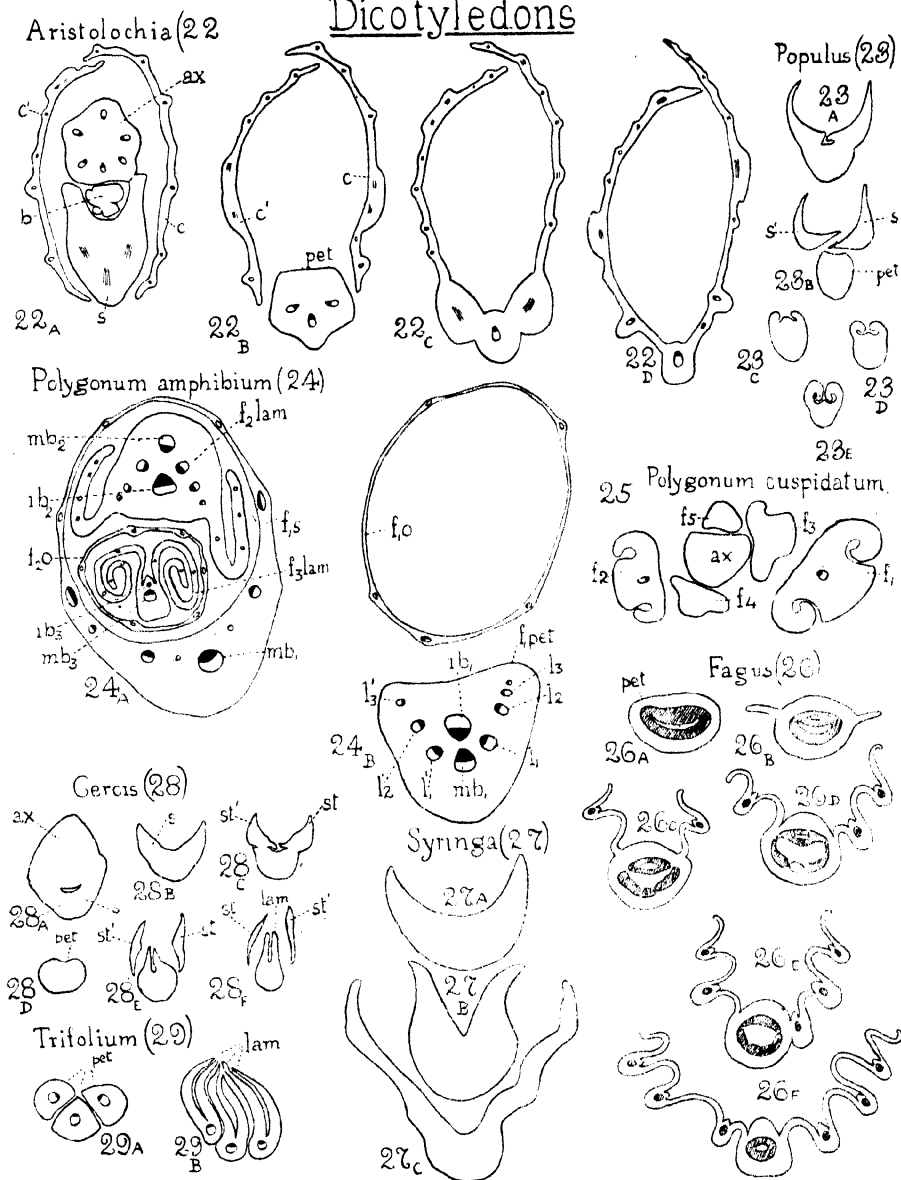
I have described, in the paper just cited, the origin of the highly complex leaf form of *Tigridia Pavonia*, Ker-Gawl.² Figs. 21 A-D of the present paper show the first plumular leaf of another species, *T. Pringlei*, S. Wats.—a case which is still more striking owing to its greater simplicity. In Fig. 21 A the sheath of this leaf is seen enclosing the terminal plumular bud (*ax.*). In Fig. 21 B dorsal invaginations are beginning to make their appearance to right and left, while in Fig. 21 C the upper limit of the sheath has been passed, and the origin of the laminations, by means of deep invaginations of the petiole, is clearly displayed. The median bundle (*m.b.*) is less conspicuous than the main laterals (*l.*₁ and *l.*₁). In Fig. 21 D the 'plicate' form is fully attained. The cotyledon sheath (*cot. s.*), which encloses the first plumular leaf, whose history we have been following, is not completely indicated except in Fig. 21 D; in Figs. 21 A, B, C, its inner epidermis alone is shown. The curious vascular arrangement in the sheath is due to the fact that the cotyledonary strand (*cot. v.b.*) doubles on itself and is thus cut twice.³

¹ Arber, A. (1921³), Figs. 1 and 2, p. 103.

² Arber, A. (1921¹).

³ Sargent, E., and Arber, A. (1915), p. 168 and Text-fig. 8.

Dicotyledons



FIGS. 22-29. Figs. 22 A-D, *Aristolochia Clematidis*, L., series of transverse sections of a single leaf from apical bud ($\times 23$); Fig. 22 A, attachment of sheathing base, *s.*, enclosing axillary bud, *b.*, to axis, *ax.*; *c.* and *c'*, bases of cordate lobes of lamina; Fig. 22 B, petiole, *pet.*; Figs. 22 C and D, lamina. Figs. 23 A-D, *Populus* sp., series of transverse sections through a very young leaf ($\times 23$); Fig. 23 A, sheathing base; Fig. 23 B, stipules, *s.* and *s'*, detached from petiole, *pet.*; Figs. 23 C-E, development of lamina. Figs. 24 A and B, *Polygonum amphibium*, L. Fig. 24 A, transverse section of apical bud passing through three leaves, *f.*₁, *f.*₂, and *f.*₃ ($\times 23$); *f.*₁*s.*, sheath of first leaf; *m.b.*₁, median bundle of first leaf; *f.*₂*lam.*, lamina of second leaf; *m.b.*₂ and *i.b.*₂, median bundle and inverted bundle of second leaf; *f.*₃*o.*, ochrea of second leaf; *f.*₃*lam.*, lamina of third leaf; *m.b.*₃ and *i.b.*₃, median bundle and inverted bundle of third leaf; Fig. 24 B, leaf 1 at a higher level ($\times 23$); *f.*₁*o.*, ochrea;

III. OBSERVATIONS ON THE ORIGIN OF THE LAMINA IN THE LEAF OF CERTAIN DICOTYLEDONS.

Aristolochia.

I have studied the structure of the young leaf of *Aristolochia Clematitis*, L., because the cordate form of the lamina and the isolation of the bundles in the axis and petiole by broad medullary rays produce a certain similarity of type to some of the Monocotyledons we have been considering. The leaf at the point of detachment from the axis (*ax.*, Fig. 22 A) has a sheathing base (*s.*) enclosing the axillary bud (*b.*). The three main leaf-bundles pursue an oblique course at this level, since they are close to their points of egress from the axis. The cordate lobes at the base of the lamina (*c.* and *c'*.) are, in the section figured, cut on either side of the leaf-base. A little higher (Fig. 22 B) the petiole (*pet.*) assumes its definitive form. Higher still (Fig. 22 C) we see the passage of the petiole into the lamina, while Fig. 22 D shows the lamina itself. The ribbing of the underside recalls the slight invagination of the lower surface of the *Tamus* leaf (Fig. 18, p. 341).

Polygonum.

The leaf of *Polygonum amphibium*, L., to some extent resembles that of *Potamogeton natans* among the Monocotyledons, and for this reason I chose it for examination. Fig. 24 A shows a transverse section of an apical bud, passing through the sheathing leaf-base (*f.*₁ *s.*) of the outer leaf (*f.*₁) and the base of the lamina (*f.*₂ *lam.*) and the ochrea (*f.*₂ *o.*) of a second leaf, and also the lamina (*f.*₃ *lam.*) of a third leaf. Fig. 24 B shows the petiole (*f.*₁ *pet.*) and ochrea (*f.*₁ *o.*) into which the sheath of the first leaf seen in Fig. 24 A passes at a higher level. It will be noticed that there is a single series of bundles in the leaf-sheath, while in the petiole and lamina there is also an inverted strand (*i.b.*₂ and *i.b.*₃, Fig. 24 A, and *i.b.*₁, Fig. 24 B) opposite the median bundle. This inverted bundle arises by the fusion and subsequent branching of the lateral bundles of the sheath at a level a little below that at which the ochrea becomes free. Two of the laterals which take part in the fusion are branches of the median bundle (*m.b.*₁). On passing upwards into the limb, the bundles shown in the petiole of *f.*₁ (Fig. 24 B) meet with the following fate: *L.*₃ fuses with *L.*₂, and then

*f.*₁ *pet.*, petiole; *m.b.*, median bundle; *i.b.*, inverted bundle; *L.*₁, *L.*₂, *L.*₃, *L'*₁, *L'*₂, *L'*₃, lateral bundles. Fig. 25, *Polygonum cuspidatum*, Sieb. et Zucc., transverse section of apical bud ($\times 47$), leaf sheaths and ochreae omitted, showing five leaves at successive levels and stages of development; *f.*, petiole; *f.*₁-*f.*₃, development of coiled wings of lamina; no bundles differentiated except the median strand of *f.*₁ and *f.*₂. Figs. 26 A-F, *Fagus sylvatica*, L., series of transverse sections cut in autumn from leaves of next year's bud, showing transition from petiole (*pet.*, Fig. 26 A) to lamina (Fig. 26 F); not all from one leaf; vascular tissue shaded; xylem and phloem not distinguished. Figs. 27 A-C, *Syringa vulgaris*, L., transverse sections showing transition from extreme base of petiole (Fig. 27 A) to lamina (Fig. 27 C) ($\times 23$). Figs. 28 A-F, *Cercis Siliquastrum*, L., series of transverse sections through one leaf ($\times 23$); Fig. 28 A, attachment of sheath, *s.*, to axis, *ax.*; Fig. 28 B, sheath, *s.*; Fig. 28 C, detachment of stipules, *st.* and *st'*.; Fig. 28 D, petiole, stipules omitted; Figs. 28 E and F, development of lamina, *lam.* Figs. 29 A and B, *Trifolium repens*, L., sections through one leaf ($\times 23$) to show origin of laminae of leaflets (*lam.*, Fig. 29 B) from petiolules (*pet.*, Fig. 29 A).

($L_3 + L_2$) fuses with L_1 . A comparison of the petiole (f_1 *pet.*) in Fig. 24 B with the two laminae cut at different levels in Fig. 24 A (f_2 *lam.* and f_3 *lam.*) shows that the lamina arises as lateral marginal outgrowths from the petiole, which become coiled as they develop.

In *Polygonum cuspidatum*, Sieb. et Zucc., the development of the lamina is essentially similar. Fig. 25 shows part of a transverse section of a very young leaf-bud of this species, in which, for simplicity, the leaf-sheaths and ochreas are omitted. In the leaf f_5 the petiole is cut across; in the leaves f_4 and f_3 the lamina is seen arising as a pair of marginal wings; in the leaves f_2 and f_1 , which are each old enough to show a lignified median strand, the wings of the lamina are elongating and beginning to coil.

Populus.

Serial sections of the bud of a cultivated species of Poplar (probably *Populus nigra*, L., or a related form) show, at the base of the leaf, the stipules and petiole united into a sheath (Fig. 23 A). Higher up the stipules ($s.$ and s' .) and petiole (*pet.*) become free (Fig. 23 B). The two halves of the lamina develop—as in *Polygonum*—as outgrowths from the margins of the petiole (Fig. 23 C), which become spirally coiled (Figs. 23 D and E). But whereas the rolling is revolute in *Polygonum* it is involute in *Populus*.

Fagus.

Sections of young leaves, dissected in October from a next year's bud of the Beech, *Fagus sylvatica*, L., show that the lamina develops as a pair of flat wings, which fold in a fan-like manner as they increase in area (Figs. 26 A–F).

Cercis.

The simple, almost cordate, blade of the Judas-tree, *Cercis Siliquastrum*, L., has a sheathing base ($s.$, Figs. 28 A and B) from which stipules ($st.$ and st' .) detach themselves (Fig. 28 C). The petiole shows its characteristic form in Fig. 28 D, while in Figs. 28 E and F the halves of the lamina are seen arising from the petiole as upwardly directed outgrowths.

Trifolium.

The laminae of the leaflets of the compound leaf of *Trifolium repens*, L. (Fig. 29 B), develop as outgrowths of the partial petioles (Fig. 29 A), very much as the blades of the simple leaves of *Cercis* arise from their leaf-stalks.

Syringa.

The Lilac, *Syringa vulgaris*, L., is an example of a leaf in which the petiole (Fig. 27 A) passes almost insensibly into the lamina (Fig. 27 C).

IV. COMPARISON BETWEEN THE ONTOGENY OF THE BLADE IN MONOCOTYLEDONOUS AND DICOTYLEDONOUS LEAVES.

In the earlier part of this paper (pp. 330-43 and Figs. 1-21) I have reviewed the mode of development of a number of Monocotyledonous leaves belonging to that type—somewhat exceptional within this Class—in which there is a definite distinction between stalk and blade. In undertaking this study I have had in mind the idea that such blades are not true laminae, but represent modifications of the distal part of the petiole; and I thought that a comparison of the development of these 'pseudo-laminae' with that of the laminae of Dicotyledonous leaves might be a help in estimating what degree of validity could be claimed for this idea. It seemed to me that the severest test of the pseudo-lamina conception would be to compare the development of the Monocotyledonous leaves in question with that of Dicotyledons whose blades resemble them in type, so that a number of differences of an obvious kind would be at once eliminated. In many of those Monocotyledonous leaves which have a differentiated blade, this blade is either ovate in form (often with a tendency to the production of a cordate or auricled base), or else it is plicated in a fan-like manner. The Dicotyledons whose leaf-development has just been described (pp. 335 and 346 and Figs. 22-9) were chosen because their blades conformed to one of these types; in a few cases they were also selected for some special reason—*Aristolochia* because of Monocotyledon-like features in its anatomy, and *Polygonum* because of the resemblance in construction between the leaf of this genus, with its ochrea, and that of certain Potamogetons. The result of my observations—stated in the most general terms—is that the lamina of those Dicotyledons which I have examined arises as wing-like outgrowths from the sides of the petiole, while in some cases the petiole itself may be regarded as undergoing lateral expansion as well as winging. In certain Monocotyledons the 'lamina' arises in exactly the same way. As regards the general scheme of their development, such leaves as those of *Potamogeton* (Fig. 1, p. 331), *Hydrocharis* (Fig. 4, p. 331), *Calla* (Fig. 8, p. 336), *Rhipogonum* (Fig. 16, p. 338), and *Tamus* (Fig. 18, p. 341) are scarcely distinguishable from those of Dicotyledons. As far as these leaves are concerned, it must be admitted that the developmental evidence affords no active support to the pseudo-lamina theory; such a blade, for instance, as that of *Tamus* (Fig. 18, p. 341) recalls in its development that of *Aristolochia* (Fig. 22, p. 344), while those of *Potamogeton* (Fig. 1, p. 331) and *Aponogeton* (Fig. 3, p. 331) resemble that of *Populus* (Fig. 23, p. 344). Such negative evidence does not, however, disprove the pseudo-lamina theory, for a blade derived from a petiole in the simplest possible way—namely, by flattening, expansion, and winging—could scarcely fail to present an exact simulacrum of a true lamina.

But the uniformity of development met with in the Dicotyledons which we have described, is not paralleled among the Monocotyledons. Besides those cases to which we have just alluded, which approximate to the Dicotyledonous type, we have a number of others in which *invagination of the petiolar tissues* plays a part of greater or less importance in the formation of the blade. Such cases have a remarkably wide distribution through the Class. I have described examples in the present paper from the Palmae, Cyclanthaceae, Araceae, Liliaceae, Amaryllidaceae, Dioscoreaceae, and Iridaceae,¹ and I have illustrated the origin of the 'lamina' by invagination in the case of *Areca* (Fig. 5, p. 334), *Oreodoxa* (Fig. 6, p. 334), *Carludovica* (Fig. 7, p. 334), *Pistia* (Fig. 12, p. 336), *Veratrum* (Fig. 13, p. 338), *Smilax* (Figs. 14 and 15, p. 338), *Curculigo* (Fig. 17, p. 341), *Dioscorea* (Fig. 19, p. 341), and *Tigridia* (Fig. 21, p. 341). The process is perhaps most strikingly displayed in the Palms and Irids; in many cases it produces a 'plicate' form of blade. How fundamentally this 'plication' differs from the genuine folding met with in some Dicotyledons, will be realized on comparing Figs. 5 A-E, p. 334 (illustrating the formation of the plicate limb of the Palm, *Areca sapida*, by alternating invaginations which penetrate between the vascular strands of the petiole), with Figs. 26 A-F, p. 344 (illustrating the development of the Beech-leaf, *Fagus sylvatica*, in which the two halves of the lamina fold up in a delicately fan-like fashion as they increase in width). As another pair of contrasting cases we may take *Crocus* and *Polygonum*; in the *Crocus*-leaf² a couple of dorsal invaginations finally produce a revolute vernation closely similar to the arrangement of the coiled lateral wings of the leaf of *Polygonum* (Fig. 24, p. 344).

Though I have not observed invagination as a main factor in lamina development either in the Helobieae or in the Araceae (except *Pistia*), yet in the cases which I have examined from these groups in which the leaves are definitely auricled at the base (*Sagittaria* of the Alismaceae, *Calla*, *Arum*, and *Epipremnum* of the Araceae) I have found that the auricles are detached by the penetration of a deep groove, which should perhaps be classed with the invaginations met with elsewhere among Monocotyledonous leaves. These grooves in *Sagittaria* start from the dorsal surface (see arrows in Figs. 2 D and H, p. 331), whereas in *Calla*, *Arum*, and *Epipremnum* they penetrate into the leaf from the ventral side (Figs. 8 E, 9 D, and 11 B, p. 336).

From the comparison which we have instituted between the development of Monocotyledonous and Dicotyledonous laminae, I think we may conclude that, although in a number of Monocotyledonous leaves 'blade' development proceeds on lines closely similar to those followed in the case of the true laminae of Dicotyledons, yet in some cases whole families (e.g. Palmae) and in other cases individual genera within a family

¹ The Gramineae, which will be described in a later paper, may be added to this list.

² Arber, A. (1921¹), Figs. 56 and 57, p. 324.

(e.g. *Veratrum* and *Smilax* in the Liliaceae) owe the production of their 'blades' to invagination of the distal region of the petiole or sheath, a process which does not—so far as my observations go—occur as a prime factor in the leaf development of Dicotyledons. These cases of invagination are so numerous and so widely scattered through the Class as to suggest that they have a definite phylogenetic significance, and that they represent a morphological tendency inherent in the group as a whole. I think we are justified in regarding the part played by invagination in the development of the blades of Monocotyledons as offering some confirmation of the view that these organs are pseudo-laminae.

V. CLASSIFICATION OF THE 'BLADES' OF MONOCOTYLEDONOUS LEAVES.

I propose, in the present section of this paper, to assume the truth of the phyllode theory, and to attempt a classification of the pseudo-laminae of Monocotyledonous leaves, based upon the factors to which they owe their development from the distal region of the petiole (or sheath). These factors are :

- (i) flattening ;
- (ii) expansion, associated with separation of bundles ;
- (iii) formation of wings or keels, which may be marginal only, or which may involve other regions of the leaf ;
- (iv) invaginations, which may be single or numerous, and which may penetrate into the leaf from either the dorsal or ventral surface, or both.

The ultimate form of the 'blade' depends, in the first instance, upon the form of the petiole from which it is derived, and, secondly, upon the developmental factor (or combination of factors) responsible for blade formation. On this basis the pseudo-laminae of Monocotyledons may be grouped as follows, according to their mode of development:—

1. From a petiole of more or less radial structure :

(a) By flattening and expansion alone, so that radial anatomy is retained.

(i) distal region of petiole only involved, e.g. Pontederiaceae.¹

(ii) whole petiole involved, e.g. *Allium victorialis*.²

(b) By flattening and expansion, so that radial anatomy is retained, associated with dorsal and ventral invaginations, e.g. *Pistia*³ (Fig. 12, p. 336).

(c) By invaginations, associated with expansion and winging ; the invaginations, though morphologically dorsal, penetrate the petiole laterally ;

¹ Arber, A. (1918), pp. 489-91 and Fig. 23-30.

² Arber, A. (1920³), Fig. 25, p. 457.

³ Arber, A. (1919¹).

practically the whole petiole is involved; e.g. various Iridaceae, such as *Cypella*.¹

2. From a dorsiventral petiole, or the distal region of a leaf-sheath:

(a) Mainly by expansion, e.g. *Rhipogonum* (Fig. 16, p. 338).

(b) By winging associated with expansion, e.g. *Hydrocharis* (Fig. 4, p. 331), *Aponogeton* (Fig. 3, p. 331).

(c) By both dorsal and ventral invaginations associated with expansion, e.g. Palms (Figs. 5 and 6, p. 334), *Carludovica* (Fig. 7, p. 334), *Vcratrum* (Fig. 13, p. 338), *Curculigo* (Fig. 17, p. 341).

(d) By numerous ventral invaginations, e.g. *Psamma*, and other Gramineae.²

(e) By a single ventral invagination associated with expansion and winging, e.g. *Smilax* (Figs. 14 and 15, p. 338).

(f) By numerous dorsal invaginations, associated with expansion and winging, e.g. *Dioscorca* (Fig. 19, p. 341).

(g) By two dorsal invaginations, associated with expansion and winging, e.g. *Crocus*.³

It should be noted that in the classification just outlined there is no hard-and-fast line between many of the subdivisions; the value of such a grouping lies, rather, in the picture which it offers of the great range of final form produced from the distal region of the petiole (or sheath) by the interaction, in varying proportions, of a limited number of developmental factors. The most potent of these is invagination, and it is to the very various aspects under which invagination presents itself that the remarkable series of different forms assumed by the Monocotyledonous pseudo-lamina is mainly due.

VI. SUMMARY.

As the result of a comparative study of blade development in the leaves of Monocotyledons (pp. 330-43 and Figs. 1-21) and Dicotyledons (pp. 344-6 and Figs. 22-9) it is shown that while the 'blade' in some Monocotyledons follows a course of development indistinguishable from that of certain Dicotyledons, yet in other Monocotyledons a factor is involved which appears to play no part in the development of the leaves of Dicotyledons—namely, invagination of the tissues belonging to the petiole or sheath (pp. 347-9). A classification of the 'laminae' of Monocotyledons is suggested, based upon the factors concerned in their development. This classification brings out the fact that the great range of leaf form met with in these

¹ Arber, A. (1921¹), Fig. 50, p. 521.

² These cases will be described in a later paper on the Glumiflorae.

³ Arber, A. (1921¹), Figs. 56 and 57, p. 324.

'laminae' depends primarily upon the protean forms which invagination is capable of assuming (pp. 349-50). It is concluded that the wide distribution among Monocotyledons of 'blade' formation by invagination of the distal region of the petiole or sheath, confirms the view that the leaf 'blades' of this class are pseudo-laminae.

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Development of Root System of Wheat in Different Kinds of Soils and with Different Methods of Watering.

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With three Figures in the Text.

A STUDY of the development of the root system in different kinds of soil and under varying conditions of manuring, watering, and cultivation, is of considerable importance in the Punjab (India), especially where the crops have to depend mainly on artificial irrigation.

The experiments to be described were confined to growing wheat in pots only. The plants were grown in two sets. The first set, A, was sown on the 21st of April and the second, B, on the 31st of May.

Set A. Six pots—8½ in. × 11 in. each—of each of the soils referred to below were taken. Three of these were watered from above as usual, and three were watered from below as explained later on. These thirty pots were divided into three lots of ten similar pots each, from which the wheat roots were washed out at three different stages of the growth of the plants, i.e. 35, 55, and 88 days after sowing. The pots were numbered as follows:

- | | |
|--|---------------------|
| 1. Heavy Rothamsted soil | watered from below. |
| 2. „ „ | watered from above. |
| 3. 25 per cent. sand + 75 per cent. soil No. 1 | watered from below. |
| 4. „ „ „ | watered from above. |
| 5. Brick-powder | watered from below. |
| 6. „ „ „ | watered from above. |
| 7. 50 per cent. sand + 50 per cent. soil No. 1 | watered from below. |
| 8. „ „ „ | watered from above. |
| 9. Sand lying on 3 in. of farm-yard manure | watered from below. |
| 10. „ „ „ | watered from above. |

Sowing.—Seeds of wheat (Red Standard) were graded between 0.06 and 0.07 grm. Two seeds were sown in the centre of each pot, and as the seedlings came up they were reduced to one in each pot on the 9th of May. They were watered as required, the same amount of water being applied to all the pots.

Watering from below.—Two small earthenware pots—2½ in. × 2 in. each with a hole at the bottom—were placed in each big pot with their tops level with the surface of the soil, so that they were equidistant from the centre of the big pot. When water was poured into these small pots it passed into the soil without disturbing the surface.

Washing.—The method of washing the roots was the same as described by Brenchley and Jackson.¹ They were washed on May 26, June 15, and July 18. The dry weights of roots and shoots are given in Table I, and the photograph of the second lot (June 15) is given in Fig. 2.

Table I shows the relative condition of the plants 35, 55, and 88 days old in all the ten pots. After thirty-five days the plants watered from below could be arranged in the following order of development, Nos. 9, 1, 3, 7, and 5, and those watered from above, Nos. 10, 2, 4, 8, and 6. The roots in Nos. 9 and 10 reached the zone of manure through the layer of sand; in Nos. 1 and 2 they spread well in the heavy soil, but remained near the surface. In the lighter soil they penetrated deeper. In the brick-powder the growth was very poor. Comparing the two methods of watering—other conditions being similar—all the plants watered from below were ahead of those watered from above. The ratios between the roots of plants watered in different ways in the same soil show that at this stage the difference is well marked in lighter soils, and it becomes progressively less so in passing from the lighter to the heavier soil.

After fifty-five days the plants watered from below could be arranged in the order Nos. 9, 3, 1, 7, and 5, and those watered from above Nos. 10, 2, 4, 8, and 6. At this stage the previous order has changed and the plant No. 3 in the soil with 25 per cent. sand has gone ahead of the plant No. 1 in the heavy soil. The plants in all the soils watered from below are still in a better condition than those watered from above, but the difference is more marked in the heavier soil than in the lighter one (*vide* curve, Fig. 1).

After eighty-eight days the plants in the pots 9, 7, and 3 were observed to have turned pale. On washing it was found that the roots had curled round and round in thick layers at the bottom of the pots. Moreover, the colour of the roots was found to have turned yellow, possibly on account of excessive heat. The relative condition of the roots was the same as in the early stage; but the difference in those watered from below and from above was still more marked in the heavier soil than in the lighter one (*vide* curve, Fig. 1, roots). The growth of the plants Nos. 9, 7, and 3 seemed to have suffered a good deal as compared with that of the plants Nos. 10, 8, and 4. The rate of growth of these plants had become slower than that in the case of corresponding plants watered from above. After a certain stage in growth these plants appear to have suffered, probably for want of space.

¹ Brenchley, W. E., and Jackson, V. G. (3921): Root Development in Barley and Wheat under Different Conditions of Growth. *Ann. Bot.*, xxxv, p. 535.

TABLE I. Dry weights of roots, shoots, &c., in Set A.

Pot No.	Soil.	How watered.	May 26—35 days after sowing.						June 15—55 days after sowing.						July 18—88 days after sowing.					
			Dry wt. of shoots.	Dry wt. of roots.	Total dry wt.	% root to total dry wt.	Grm.	Root ratios, i.e. watered below, watered above.	Dry wt. of shoots.	Dry wt. of roots.	Total dry wt.	% root to total dry wt.	Grm.	Root ratios, i.e. watered below, watered above.	Dry wt. of shoots.	Dry wt. of roots.	Total dry wt.	% root to total dry wt.	Grm.	Root ratios, i.e. watered below, watered above.
1	Heavy	Below	0.47	0.23	0.70	32.86	0.47	1.09	0.38	0.21	0.59	35.59	0.38	1.09	0.63	0.94	1.57	39.49	0.63	2.13
2	Do.	Above	0.38	0.21	0.59	35.59	0.38	1.09	0.38	0.21	0.59	35.59	0.38	1.09	0.63	0.94	1.57	39.49	0.63	2.13
3	1 sand	Below	0.43	0.22	0.65	33.85	0.43	1.47	0.43	0.22	0.65	33.85	0.43	1.47	0.76	0.88	1.64	43.94	0.76	1.38
4	Do.	Above	0.30	0.15	0.45	33.33	0.30	1.47	0.30	0.15	0.45	33.33	0.30	1.47	0.76	0.88	1.64	43.94	0.76	1.38
5	Brick-powder	Below	0.12	0.11	0.23	47.83	0.12	1.22	0.12	0.11	0.23	47.83	0.12	1.22	0.70	0.46	1.16	39.66	0.70	1.31
6	Do.	Above	0.10	0.09	0.19	47.37	0.10	1.22	0.10	0.09	0.19	47.37	0.10	1.22	0.41	0.35	0.76	46.05	0.41	1.31
7	1 sand	Below	0.30	0.20	0.50	40.00	0.30	1.54	0.30	0.20	0.50	40.00	0.30	1.54	0.95	0.61	1.56	44.67	0.95	1.07
8	Do.	Above	0.17	0.13	0.30	43.33	0.17	1.54	0.17	0.13	0.30	43.33	0.17	1.54	0.43	0.26	0.69	41.45	0.43	1.07
9	Sand with 3 in. F. Y. M. at the bottom	Below	0.88	0.25	1.13	22.12	0.88	1.66	0.88	0.25	1.13	22.12	0.88	1.66	13.82	8.74	22.56	38.74	13.82	1.61
10	Do.	Above	0.62	0.15	0.77	19.48	0.62	1.66	0.62	0.15	0.77	19.48	0.62	1.66	18.44	5.43	23.87	22.75	18.44	1.61

TABLE II. Dry weights of roots, shoots, &c., in Set B.

Pot No.	Soil.	Flow watered.	July 14—45 days after sowing.					July 28—59 days after sowing.					August 4—66 days after sowing.				
			Dry wt. of shoots.	Dry wt. of roots.	Total dry wt.	% root to total dry wt.	Root ratios, i.e. watered below. watered above.	Dry wt. of shoots.	Dry wt. of roots.	Total dry wt.	% root to total dry wt.	Root ratios, i.e. watered below. watered above.	Dry wt. of shoots.	Dry wt. of roots.	Total dry wt.	% root to total dry wt.	Root ratios, i.e. watered below. watered above.
1	Sand underlaid with F. Y. M.	Below	1.15	0.32	1.67	31.14	0.61	4.20	1.10	5.30	20.75	0.91	11.18	2.30	13.48	17.06	0.97
2	Do.	Above	2.04	0.85	2.89	29.41		6.62	1.21	7.83	15.45		13.94	2.38	16.32	14.58	
3	Sand	Below	0.14	0.09	0.23	39.13	0.82	0.21	0.09	0.30	30.00	0.75	0.40	0.12	0.52	23.08	0.86
4	Do.	Above	0.18	0.11	0.29	37.93		0.32	0.12	0.44	27.27		0.46	0.14	0.60	23.33	
5	Brick-powder underlaid with F. Y. M.	Below	0.29	0.13	0.42	30.95	1.86	0.67	0.15	0.82	18.29	1.50	0.85	0.19	1.04	18.27	2.37
6	Do.	Above	0.13	0.07	0.20	35.00		0.38	0.10	0.48	20.83		0.42	0.08	0.50	16.00	
7	Brick-powder	Below	0.13	0.12	0.25	48.00	0.92	0.19	0.05	0.24	20.83	0.62	0.50	0.10	0.60	16.65	1.11
8	Do.	Above	0.22	0.13	0.35	37.14		0.28	0.08	0.36	22.22		0.21	0.09	0.30	30.00	
9	25% sand + 75% heavy soil underlaid with F. Y. M.	Below	3.85	0.69	4.54	15.19	0.63	10.60	1.70	12.30	13.82	1.07	12.99	1.75	14.74	11.87	0.91
10	Do.	Above	4.14	1.10	5.24	20.99		11.45	1.59	13.04	12.20		13.76	1.93	15.69	12.30	
11	25% sand + 75% heavy soil	Below	2.10	0.70	2.80	25.00	0.77	6.98	1.83	8.81	20.77	1.19	9.93	2.25	12.18	18.47	1.41
12	Do.	Above	2.70	0.91	3.61	25.21		7.56	1.54	9.10	16.92		10.11	1.60	11.71	13.67	

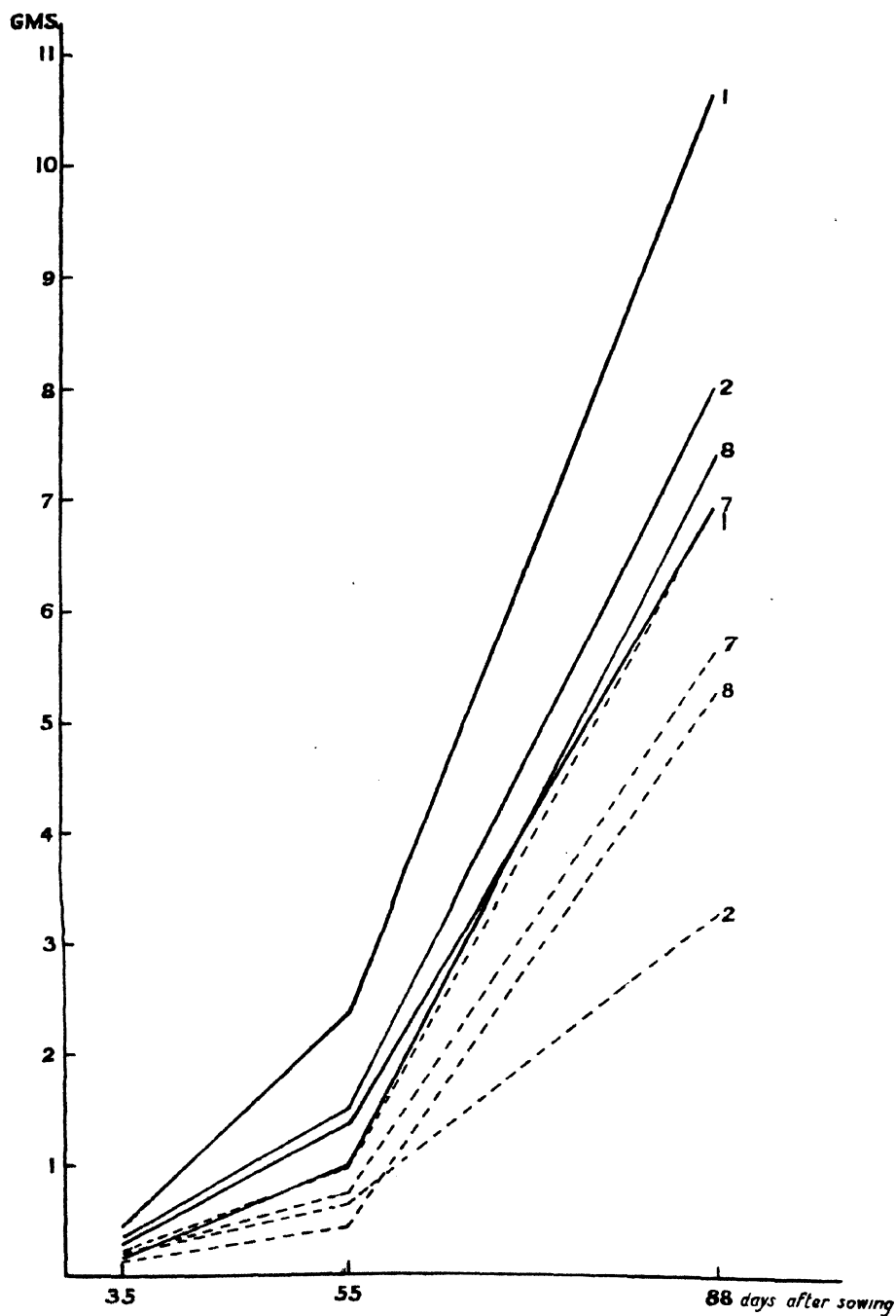


FIG. 1. Curves showing dry weights of wheat plants at three stages of growth in heavy and light soils, with different methods of watering. (1) Heavy soil watered from below; (2) heavy soil watered from above; (7) light soil watered from below; (8) light soil watered from above. — = shoot. = root.

The number of the shoots and leaves of the plants in pots watered from below was observed to be invariably greater than that of plants in corresponding pots watered from above.



FIG. 2. Set A. Wheat plants on June 15, i. e. 55 days after sowing. Order: 1, 3, 5, 7, 9, 2, 4, 6, and 10, from left to right.

Set B. Soils.—Three kinds of soil were used. (1) Pure sand, (2) brick-powder, (3) a mixture of 25 per cent. sand and 75 per cent. Rothamsted soil.

Twelve pots of each soil were taken, six watered from below and six watered from above, and in half of them 3 in. of farm-yard manure was placed at the bottom of the pots. Thus there were three lots of twelve pots each, numbered as shown in Table II.

Sowing.—Seeds were graded between 0.05 and 0.06 grm. Seeds were sown on the 31st of May in each pot— $6\frac{1}{2} \times 15$ in. Only one small earthenware pot was placed in each of the big pots, for watering from below.

Washing.—Washing was done as before at three stages of growth, i. e. 45, 59, and 66 days after sowing. The dry weights of roots and shoots are given in Table II and the photograph of the first lot at forty-five days in Fig. 3.

This set grew under somewhat abnormal conditions, as the weather was comparatively warm. The seeds took fourteen days to germinate in brick-powder, ten days in sand, and one week in loamy soil. A variety of spring wheat was used, as opposed to winter wheat in set A.

As might be expected, owing to the abnormal weather conditions, at all stages the difference in the condition of the plants watered from below

and above was less marked than in the first experiment. The difference in the root system in the three kinds of soils, with manure and without, is of interest. In the loamy soil it was significant, but decreased with the growth. In sand it was very large. In brick-powder the manure did not seem to have had any effect. In sand without manure there was considerable spreading of the roots in the deeper layers, which was apparent even in the early stage. The plants do not appear to thrive in brick-powder under any conditions.

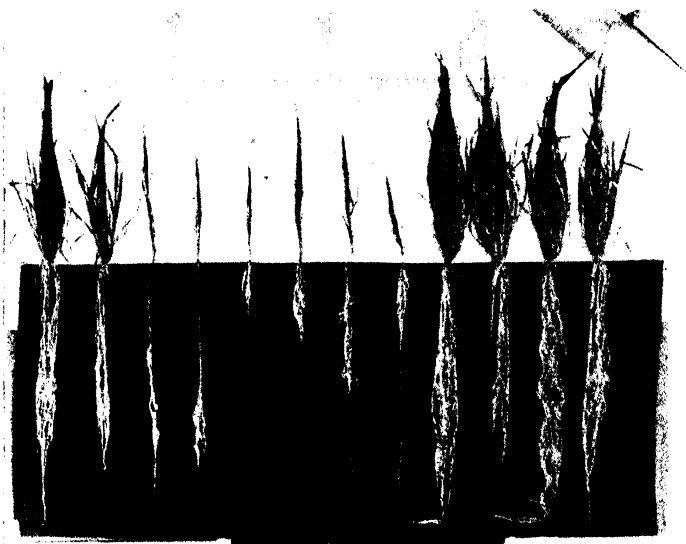


FIG. 3. Set B. Wheat plants on July 14, i.e. 45 days after sowing. Order: 2, 1, 4, 3, 6, 5, 8, 7, 10, 9, 12, 11, from left to right.

At the time of washing the roots of the plants great difference was observed in the texture of the soil in the pots watered from below and in those watered from above. In the latter case the soil was compact to a depth of five or six inches from the surface, whereas the soil underneath was in a loose condition, showing that the movement of free water was confined to the top 6 in. and that the capillary movement of soil moisture was much better in this part than in the loose soil below. In the former case the soil was loose to a depth of 2 in. or 3 in. from the surface, but was compact below. The growth of the roots is much better in the compact zone on account of better movement of soil moisture. This explains, to a considerable extent, why the growth of the roots and shoots is greater when the pots are watered from below.

It is recognized that the observations here described are of a somewhat preliminary nature; but circumstances prevented the work being carried farther.

SUMMARY.

1. Wheat plants in pots show better growth when watered from below than when watered from above. The difference is greater in light soil in the early stages of growth ; but it is more marked in heavy soil in the later stage of growth.

2. Under the experimental conditions the development of root and shoot was best in pure sand provided it was supplied with an adequate amount of water and was underlaid by a layer of farm-yard manure.

3. The growth of wheat is better in a mixture containing 25 per cent. sand and 75 per cent. Rothamsted soil than in pure Rothamsted soil or in a mixture of 50 per cent. sand and 50 per cent. Rothamsted soil. Moreover, wheat plants do not grow well in brick-powder even when underlaid with a layer of farm-yard manure ; observations here put forward are of a somewhat preliminary nature.

In conclusion, I wish to express my sincere thanks to Dr. W. E. Brenchley for her help during the investigations and for facilities provided for conducting the experiments.

Further Observations on the Transpiration, Stomata, Leaf Water-content, and Wilting of Plants.

BY

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With three Figures in the Text.

THE results of investigations on the transpiration stream and the water-supply of plants have directed attention to the phenomenon of wilting and to its relation to environmental conditions of soil and atmosphere. The essential turgidity of the plant is maintained by virtue of the capacity of the tissues to maintain within them a sufficiency of water. The turgor equilibrium in the cell system is not a simple one. It is complicated by the fact that it is not a static balance, but the water is in a state of continual motion, passing from cell to cell with the upward flow of the transpiration stream. Thus, unless the movement of water is closely regulated, so that as the water leaves a cell it is replaced by the absorption of an equivalent quantity, there must result fluctuations in the state of the turgidity of the cell. Inability of the cell to obtain sufficient water to counterbalance the loss by transpiration or translocation results in wilting. It is evident at the outset therefore that the turgidity of the plant is influenced by the factors governing water loss and water absorption, viz. the atmospheric evaporating power and the quantity and rate of translocation of water through the soil and through the plant. Much attention has therefore been focused on the questions of wilting coefficients and available soil moisture. Tentative efforts have also been made to interpret the wilting process in terms of the functions of the plant. Beyond recognition of the fact that wilting involves a decrease in water-content, little progress has been made in the analysis of the phenomenon. Distinction has been drawn between the early stages of the process, 'incipient wilting', and the later stages, known simply as 'wilting'. It is not at all clear how it is possible to determine the line of demarcation between these two stages of the process, and there is, moreover, no evidence that there is any reason for such demarcation. It may be urged that 'wilting' implies the visible collapse of the leaf, whilst 'incipient

wilting' consists merely of a decrease in leaf water-content without visible collapse. Such a criterion, susceptible as it is to personal error, can hardly be admitted as a help to a real analysis of the problem. Consequently, in the present paper, the term 'wilting' simply denotes a decrease in water-content.

The concept of 'permanent wilting' has been employed by Shive and Livingston in a study of the wilting phenomena. A plant is said to be permanently wilted when transference to an atmosphere saturated with water vapour fails to revive it within twenty-four hours. The difficulty and labour of the determination of the permanent wilting-point are self-evident, and have militated against the use of this method. Bakke (1, 2) has preferred to adopt changes in the transpiration rate as criteria of the progress of wilting. He used Stahl's cobalt chloride method, as modified by Shreve and Livingston, for determining transpiration rate, and found that as the plant wilts this rate falls steadily to a minimum, which is maintained for a longer or shorter period, depending upon the species. Following this period the transpiration rate rises rapidly to a maximum before the final decrease accompanying the drying out of the plant. Bakke attributes this sudden increase in transpiration rate to the sudden rupture of the water columns in the plant, and the point at which this occurs is considered to be the permanent wilting-point. No evidence has been adduced, however, to show that this point coincides with, or indeed has any relation to the permanent wilting-point as defined by Shive and Livingston. It appears that experimental demonstration of the sudden rupture of the water columns in the plant, postulated by Bakke, would prove difficult. The permanent wilting-point of Shive and Livingston may be defined as the stage during the wilting process at which the process of diminution of cell water-content, hitherto reversible, becomes irreversible. It is of course conceivable that the passage of the wilting process from the reversible to the irreversible stage may be accompanied by some sudden internal change such as that postulated by Bakke, but at present there appears to be no evidence of the occurrence of a sudden and complete rupture of the water columns such as might be responsible for a sudden increase in the rate of transpiration. The presence of air gaps in the water columns at certain seasons of the year is undoubted. Dixon (10) has dealt with this point at some length, and Farmer (11, pp. 245-7) has traced the gradual disappearance of air from the wood in autumn as indicated by the increase of density. This increase was shown to spread gradually from the basal shoots to those situated nearer the apex of the tree. The bubbles of air appear again in the wood during the summer as the result of the tension set up by the evaporation of water from the leaves, but at present there is no experimental evidence known to the present writer to show whether the appearance of bubbles is a sudden or a gradual process. *A priori*, one would expect that the quantity of air

present in the path of the transpiration stream would gradually increase as the summer advanced. It is true that the root system steadily develops, but not only does the total area of the transpiring surface of the plant steadily increase, but in addition the atmospheric evaporating power also gradually increases and soil moisture diminishes. All these factors would tend to increase the tension on the water columns. The 'actual wilting process may obviously be regarded as the result of a further extension of this general seasonal tendency of the environment. As the plant passes from a state of turgor to the wilted condition, there is no reason to expect that at any particular stage all the water columns are suddenly ruptured. It is more natural to suppose that the replacement of water by air is a gradual process during wilting, as during the ordinary seasonal changes, the water columns being severed one by one with the increasing tension, until the number remaining unbroken cannot supply the leaves with sufficient water to keep the plant alive. This view is susceptible to experimental test by means of determinations of density changes during wilting, on the lines of Farmer's observations. It is hoped shortly to carry out an investigation of this point.

Having demonstrated the fact that during wilting the plant reaches a stage at which its transpiring power ceases to diminish and suddenly increases, Bakke (2) sought to discover possible explanations other than his theory of the rupture of the water columns. He attempted to establish a correlation between the increase of transpiring power at the 'permanent wilting' stage and the opening of the stomata which Darwin (7, 8) and Darwin and Pertz (9) showed to occur on wilting. Darwin's original methods estimate water loss and not stomatal aperture, but the porometer method of Darwin and Pertz was more direct and confirmed the previous results. Lloyd, using his alcohol method, failed to demonstrate the stomatal opening on wilting, but Laidlaw and Knight (16), with the porometer, obtained results¹ in agreement with those of Darwin and Pertz. Bakke (2) failed to find any appreciable stomatal opening during wilting, but rightly insists that it is important that the question of stomatal influence on transpiration during wilting should be settled.

It may be considered significant that investigators who have demonstrated the occurrence of stomatal opening during wilting have all used the porometer method, indicating that the result obtained might be due to some inherent quality of the method. It has been shown, however (16), that there is no reason to suppose that the method is at fault in this particular case. A study of the limitations of the method has already been made (14), but it is perhaps desirable to mention some remarks of Gray and Peirce (12) upon the method. They state that 'Obviously, stomata within a porometer are shut off from both light and air'. It is true that the

¹ For a consideration of the explanation of this phenomenon, see the paper referred to.

stomata on that ring-shaped portion of the leaf which is covered by the luting material which attaches the leaf to the apparatus, are shut off from light from below and from external air, but these are not the stomata whose changes are being measured. The porometer does not deal with the stomata covered by the luting material, but with those enclosed by the leaf-chamber and those on the rest of the leaf, and it is apparently the stomata enclosed by the leaf-chamber which are referred to as being shut off from both light and air. With a clear glass leaf-chamber it is true that some of the reflected light from below is prevented from reaching the leaf, but the diffuse light transmitted through the leaf is unaffected. Further, in a porometer experiment, the stomata, far from being shut off from air, are given freer access to it, in that air passes through the leaf, under the influence of the suction applied, far more rapidly than it would normally do by diffusion. There are, however, grounds for an objection to the porometer method which has not yet been urged. Neger (18) has distinguished between two definite types of leaves. In one type the intercellular spaces are in free communication throughout the leaf and in the other type this intrafoliar communication is interrupted, the system of spaces being divided into more or less numerous units. The former type of leaf Neger calls homobaric and the latter heterobaric. Plainly it would be theoretically possible to attach a leaf-chamber to a heterobaric leaf in such a manner that it would be impossible to draw any air through at all, even though the stomata were wide open. It is evident that in comparing stomata of different plants by the porometer method, it is necessary to consider the condition of the intercellular spaces, a precaution which has already been advocated by the writer (14).

As the result of his experiences with the determinations of stomatal behaviour during wilting (16), the present writer felt convinced that the rise of transpiring power which Bakke found to accompany permanent wilting, could not possibly be associated with the stomatal opening first demonstrated by Darwin's experiments. At the outset the time factor appears to preclude the possibility of any such correlation. Thus, the rise of transpiring power referred to by Bakke is considered to be closely associated with the point at which the wilting process becomes irreversible. This point was reached in practice *some days* after the transpiration rate had begun to fall as the result of insufficient supply of water to the leaves. On the other hand, Darwin and Pertz, and Laidlaw and Knight, have given the periods which elapse, under different conditions, between the cessation of the supply of water to the leaves and the attainment of the maximum stomatal aperture consequent upon wilting. The longest period recorded by Darwin and Pertz is about 90 minutes, whilst the periods quoted by Laidlaw and Knight varied from 9 to 35 minutes. It is recognized, of course, that comparison of mere time intervals in a matter of

physiological correlation is inadmissible. The duration of the time interval must be determined by such factors as the rate of transpiration and the amount of water in the plant. It is to be expected that the time interval will be shorter for a single leaf severed at the petiole than for a leaf attached to a severed shoot, the stem of which could supply water to the leaves longer than the petiole alone. No real comparison is permissible which does not take into consideration the physiological state of the plants in the two cases, but in the present case the disparity between the two intervals is significantly large.

Again, the duration of the period of increased transpiring power appears excessively long when compared with the short time occupied by the opening movement of the stomata.

In view of these discrepancies and of the importance of the question whether stomatal opening could be held to account for the increase of transpiring power accompanying permanent wilting, the writer felt that further experiments were desirable in order to obtain more complete information with regard to the significance of the wilting process. The trend of the work has already been indicated in the writer's summary of transpiration work (15).

The first investigation undertaken was the determination of the change of transpiration rate accompanying the stomatal opening caused by wilting. Darwin (7, 8) in effect determined transpiration rate changes during wilting, and Darwin and Pertz determined stomatal changes, but there has been until now no attempt made to determine the two simultaneously for the same plant. In the present investigations plants were caused to wilt under controlled conditions, and observations were made at short intervals of stomatal aperture, transpiration and evaporation rates, and of environmental conditions such as temperature, relative humidity, and wind velocity. Since the whole wilting period during which observations were made was usually of less than two hours' duration, and since very frequent observations were necessary, the technical details of the experiment were not simple, and therefore merit some description.

Detached shoots, set up in potometers, were used in this work, the usual precautions being observed in severing the shoots from the plants. Potometers were used in preference to potted plants in order that observations could be made on the rate of water absorption by the stem as well as on the rate of water loss. In this way it was possible to detect any changes in the water-content of the plant during the preliminary period before the water-supply to the stem was intentionally cut off. The potometers were placed in the air flue previously described (3), by means of which the movement of the air passing over the plant could be maintained at a constant velocity. Throughout the experiments the velocity of the air stream has been seven metres per minute. Owing to the short duration of the experi-

ment it was found possible to maintain the temperature constant within reasonable limits, but, as the humidity was variable, determinations were made of the evaporating power of the air by means of a form of paper atmometer which has been found satisfactory for the evaporation rates normally encountered in south-east England. It consists (see Fig. 1) of a circular piece of filter-paper (A), to the centre of which is attached, with sealing-wax or shellac, a small glass cup in the form of a thistle funnel (C), such as is used in porometer work. The filter-paper is supported at its edge by a wire frame (B) which is kept in position by wire stays fixed to the glass cup. The cup and its stem are filled with water and the stem is passed through the rubber stopper of a bottle which acts as a water reservoir and at the same time supports the cup and the paper. As water evaporates from the paper, which must be completely wetted at first, it is replaced from the reservoir, and it is found that no drying out occurs at the

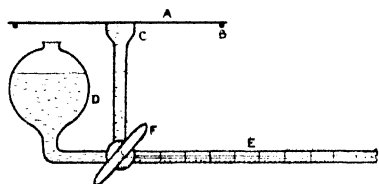


FIG. 1. Type of paper atmometer used in transpiration experiments. A. Filter-paper. B. Supporting wire frame. C. Glass cup. D. Reservoir. E. Graduated capillary tube. F. Three-way tap.

edges of the paper if the diameter of the cup is sufficiently large—about $1\frac{1}{2}$ cm. for a 9 cm. filter-paper. The paper must be of a rather loose texture or the conduction of water through it is too slow, although the use of the soft paper shortens the life of the instrument. Evaporation rates are determined by weighings. The apparatus is distinctly fragile, but easy to construct, and with careful handling

one will serve for several experiments. It has the advantage that it is very light, and naturally responds to temperature changes more rapidly than porous cup atmometers. It has proved very convenient for greenhouse work, but out of doors it would probably suffer in heavy rain or high wind. In the present experiments, owing to the short time available for observations, this paper atmometer was adapted for rapid volumetric readings in order to save the time which would be occupied by weighing. The leg of the glass cup was sealed into the side of a horizontal graduated capillary tube (E), with a reservoir (D) and three-way cock (F) attached, so that the apparatus resembled a Ganong potometer, the cup and paper replacing the plant. With this apparatus, readings of the evaporation rate could be made over successive periods, each of two or three minutes' duration. Determinations of transpiration rate of the plant and of absorption from the potometer were made eight or ten times per hour. As a weighing ordinarily takes between one and two minutes, the following procedure was adopted in order to save time. The balance was placed on the roof of the air flue, and the potometer was attached to the balance arm by a thread passing through holes in the top of the flue and the base of the

balance; on the right-hand pan of the balance were placed weights just insufficient to counterbalance the potometer. The beam was then released and its swing observed. With the loss of water by the plant the weight of the potometer eventually became equal to the weight on the right-hand pan, and the time when this occurred was noted with a stop-watch. From the right-hand pan of the balance were then removed weights totalling about 50 to 100 mg., the actual (most convenient) figure being found by experience. The beam was again set swinging and the time taken to restore the equilibrium by further water loss was recorded. Thus, instead of determining the decrease in weight during a period of known length, the time during which the plant lost a known weight of water was measured, and from this the transpiration rate was calculated. For the short period observations necessary in the present experiments this method was found more suitable than the more usual one of weighing at regular intervals. Stomatal changes were recorded automatically by the use of a modification of the recording porometer previously described (16). The modified form of the recorder has been in use for some considerable time, and, owing to its portability and reliability, it is deemed worthy of detailed description here. Air is drawn from the porometer cup through a Mariotte bottle aspirator (A) (Fig. 2) in the usual manner, a constant pressure difference being maintained. In the bottom of the aspirator is a shallow layer of mercury (B), the surface of which just touches the end of the tube (C), from which the bubbles of air emerge into the aspirator. Down the centre of this bubble delivery tube passes a mercury platinum electrode (D), the platinum point of which just fails to make contact with the surface of the mercury in the aspirator. Leads (G) from this mercury layer and from the electrode above it connect the two in series with a battery and a magnetic pen marking on the paper on a time-drum rotated by clockwork. The air enters the bubble delivery tube and passes down the annulus between it and the electrode, eventually bubbling up through the water in the aspirator. As a bubble is formed it depresses the mercury surface immediately below it. On the release of the bubble, the mercury, before coming to rest again, oscillates slightly, and in doing so makes contact momentarily with the platinum point of the electrode, thus closing the electric circuit through the battery and the pen. The pen is deflected and makes a record on the drum which indicates the release of a bubble. Thus the passage of every bubble is recorded, and

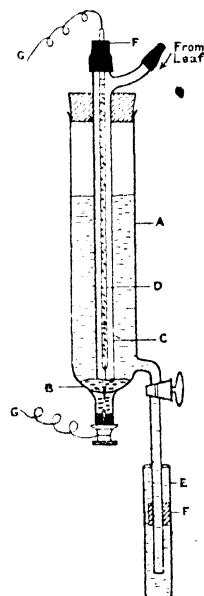


FIG. 2.
Recording porometer.

from the frequency of the bubbles can be calculated the rate at which air is being drawn through the stomata. This gives a relative measure of the size of the stomatal aperture. The success of the method depends, of course, on the equality in size of the bubbles. It is found that within reasonable limits these are of the same size as long as the apparatus remains undisturbed, although the depth of the water in the aspirator certainly does slightly influence the size. The variation in the bubble size due to this cause is very small and is also gradual, and is not a serious drawback. The regularity of the bubbles may, however, be considerably interfered with by the dripping of water from the end of the outflow tube, and for this reason it is advisable that this tube should dip into an overflow vessel (E), in order to prevent the periodical jerks on the bubble as the drops fall. During the formation of a bubble the pull on the air passing through the leaf decreases slightly as the mercury surface is depressed, returning again to its original magnitude on the release of the bubble. This change of pressure is very small, and of course the cycle of change is identical for all bubbles. Necessary adjustments of the central electrode and of the height of the outflow tube may be made by means of rubber sleeves (F).

In the experiments under consideration the recorder was placed in the flue and attached by means of T-pieces to two or three leaf-chambers, each on a different leaf. Thus an average reading of stomatal aperture on different parts of the plant was obtained. In order to avoid the necessity of disconnecting the recorder for every weighing to be made, a two feet long piece of very flexible small-bore rubber tubing was used to connect the apparatus to the leaf-chambers. This was allowed to hang in a loop, and tests showed that with a balance sensitive to about 5 mg. this length of tubing, although somewhat damping the oscillation of the beam, did not seriously affect the accuracy of the weighings. After setting up the potometers and the recording apparatus, observations were made at short intervals of temperature, relative humidity, loss of weight of the potometer, absorption of water by the plant, and rate of evaporation from the atmometer. The record of stomatal aperture was taken continuously throughout the experiment. After a period of about an hour, during which about ten sets of observations were made, it was possible by comparison of transpiration and water absorption figures to determine whether the water-content of the plant was decreasing under the conditions of the experiment. If no decrease in water-content could be demonstrated, it was assumed that the records of the preliminary period were representative of the normal progress of the functions of the plant. At this stage the water-content of the plant was experimentally diminished. This was accomplished by closing a stop-cock at the base of the potometer, thereby cutting off any further supply of water to the cut end of the stem. Observations were continued as before over a period as long as the results warranted, usually one or two hours.

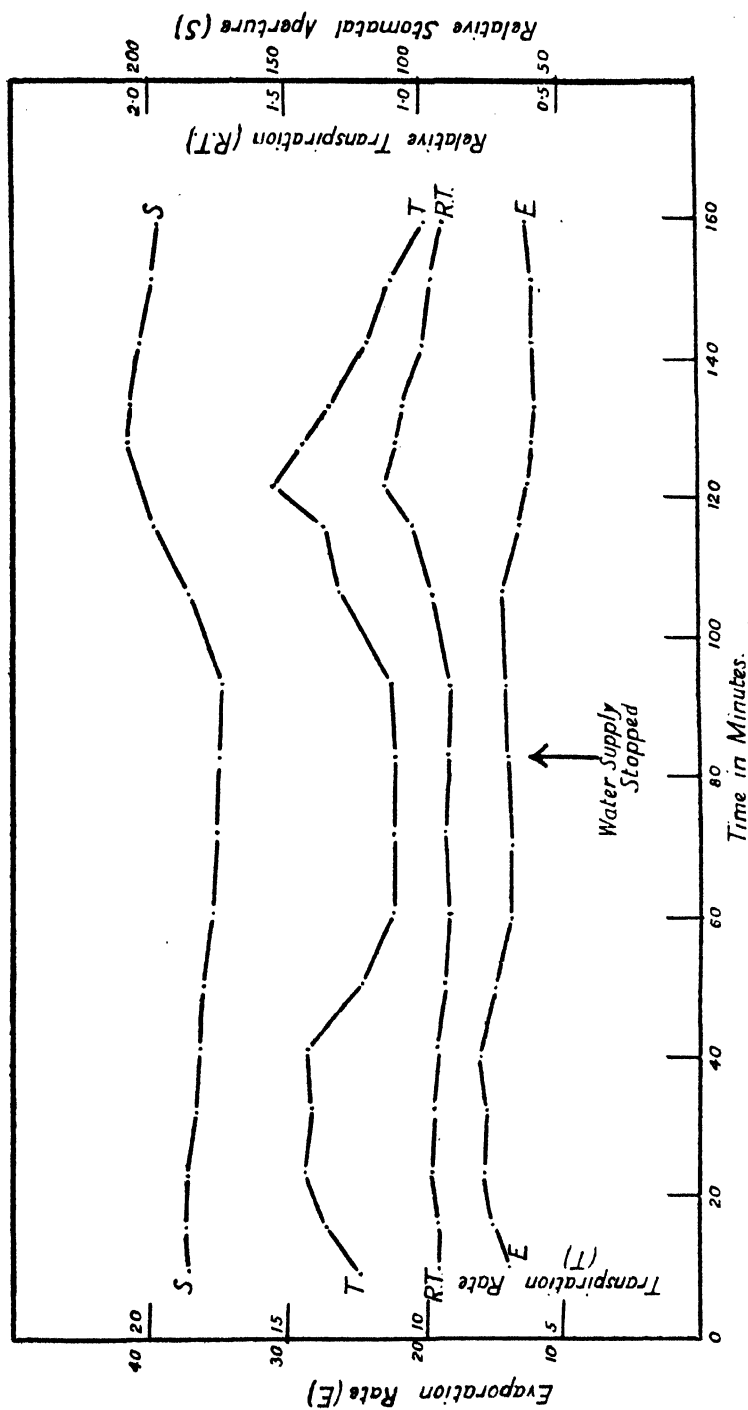


FIG. 3. Showing graphs of evaporation (E.), transpiration rate (T.), relative transpiration (R.T.), and stomatal aperture (S.), during the early stages of wilting. Although a continuous record of stomatal aperture over the whole period is available, the graph is plotted to represent the mean values for the periods covered by the values of the other graphs.

The results of such an experiment are given in Table I and Fig. 3. Comparative values for 'relative transpiration' have been obtained by dividing absolute transpiration by evaporation, although these values must be considered cautiously in the light of the work of Briggs and Shantz (4). They found that atmometers vary considerably in their response to environmental changes, and that it is not therefore permissible arbitrarily to choose any one type of atmometer for calculation of relative transpiration. Cribbs (6) also observes that the foliar transpiring index is influenced less by wind than is the porous cup atmometer, but it should be pointed out that Cribbs used the hygrometric paper method of measuring transpiration index, in which air currents are intentionally prevented from reaching the cobalt paper and also the portion of the leaf under investigation. The present writer, however, has recorded similar conclusions from the results of experiments in which the plants were subjected to air currents of known velocity (13).

TABLE I.

Showing the progress of transpiration and stomatal change during the early stages of wilting.

Period ending.		Temperature.	Relative humidity.	Evaporation rate (E.).	Actual transpiration (A. T.).	Transpiration rate (T.).	Relative transpiration (R. T.).	Absorption by stem (A.).	Progressive totals.		Stomatal aperture.
Min.	sec.	°C.	%	Mg. per min.	Mgm.	Mg. per min.	T. E.	Mg.	A.	A. T.	S.
0	8	17.7	62	—	—	—	—	—	—	—	175
9	21	17.9	62	13.6	130	12.3	0.90	130	130	130	172
16	0	17.8	63	15.0	90	13.5	0.90	110	240	220	172
23	41	17.5	65	15.4	110	14.3	0.93	100	340	330	171
32	16	17.4	65	15.3	120	14.0	0.92	130	470	450	165
41	24	17.3	66	15.7	130	14.2	0.90	130	600	580	162
51	14	17.4	68	14.6	120	12.2	0.84	140	740	700	159
61	10	17.0	69	13.5	110	11.1	0.82	390	1130	1050	153
72	1	16.7	70	13.4	120	11.1	0.83				150
82	58	16.2	69	13.7	120	11.0	0.81				148
Water-supply from potometer stopped.											
93	47	15.9	69	13.8	120	11.1	0.80	Nil.			145
106	12	16.1	69	14.1	160	12.9	0.92				167
115	53	15.8	70	12.8	130	13.4	1.05				194
121	47	15.9	71	12.2	90	15.3	1.25				208
128	7	15.7	71	11.9	90	14.2	1.19				212
133	24	15.8	72	11.8	70	13.2	1.12				210
141	52	15.8	72	11.9	100	11.8	0.99				204
151	6	15.6	72	11.8	100	11.0	0.93				195
159	16	15.8	72	12.1	80	9.8	0.81	190			

The figures in the first column represent the duration of the experiment from the commencement of observations, the zero point corresponding to 1.10 p.m. The stomatal aperture (S.) was gradually diminishing, and

with the slight fall of temperature and rise of humidity the rate of evaporation from the atmometer showed a tendency to decrease. For the first eighty minutes the graph of transpiration rate (T.) followed a course very similar to that of evaporation, and the relative transpiration graph is therefore less irregular than that of transpiration rate itself. All observations taken throughout this portion of the experiment show that the loss of water by transpiration never exceeded the amount absorbed from the potometer, and consequently there was no decrease in water-content. The stopcock on the potometer was closed after observations had been continued for eighty-three minutes, and further water-supply to the shoot was thus prevented. From this point to the end of the experiment environmental conditions, as indicated by readings of temperature, relative humidity, and evaporation rate, showed the same trend as hitherto. There was no sudden external change which could be held responsible for the changes exhibited by the plant. After a few minutes the stomatal aperture showed a sudden increase amounting to about 40 per cent. of its previous magnitude, the maximum being reached forty-one minutes after the cessation of the absorption of water by the stem. The opening of the stomata was accompanied by a simultaneous increase in the rate of transpiration, and, as the evaporation rate continued to exhibit a downward tendency, this increase of transpiration is also reflected in the relative transpiration graph.

As the decrease of water-content proceeded, the preliminary opening of the stomata gave place to a closing movement, usually associated with wilting. This closure was accompanied by a decrease in the transpiration rate, which was again greater than could be attributed to the change in the evaporation rate.

This experiment combines the features of previous experiments on transpiration rate with those on the changes of stomatal aperture of wilting plants. It shows that simultaneously with the temporary opening of the stomata which accompanies the initial stages of wilting, there also occurs a corresponding temporary increase in the rate of water loss from the leaves. In a series of sixteen experiments on two species, *Eupatorium adenophorum* and *Peristrophe speciosa*, this increase of transpiration rate has occurred on every occasion. The magnitude of the increase varies considerably, and shows a rough correlation with the magnitude of the increase in stomatal aperture, but undoubtedly there are other factors concerned in determining the extent of the increase. This point will be discussed later.

An attempt was made to correlate the variation in the magnitude of the increase with the rate at which the plant wilted. Unfortunately, different atmometers were used in different experiments, so that it was impossible to compare the evaporation rates from day to day. However, as the velocity of the air motion was constant throughout, the temperature

of the air during the various experiments gave a rough comparison of the evaporation rates and consequently of the rates of wilting, provided similar plants were used. Table II gives a list of experiments on *Eupatorium adenophorum* arranged according to the mean temperature of the air surrounding the plants during wilting. The table includes the percentage increase in size of stomata and in transpiration rate, and also the time which elapsed between the cessation of the absorption of water and the attainment of the maximum values of the two functions.

TABLE II.

Results of experiments on *Eupatorium adenophorum*, showing the progress of transpiration rate and stomatal change during wilting at different temperatures.

Experiment No.	Mean temperature during wilting. ° C.	Percentage increase of:		Period elapsing before attainment of maximum [minutes].	
		Stomatal aperture.	Transpiration.	Stomatal aperture.	Transpiration.
39	13.5	—	41	—	64
41	14.2	25	44	75	75
38	14.4	—	41	—	67
37	14.5	574	75	49	43
36	15.0	153	69	37	37
42	16.0	27	13	30	30
43	16.1	16	19	51	40
46	16.4	—	16	—	38
35	17.4	44	37	31	27
31	18.4	—	71	—	26
32	18.8	—	9	—	21
33	19.0	—	39	—	18
29	—	—	10	—	11

There is apparently no correlation between the temperature of the air in which wilting occurs (i. e. presumably the velocity of the wilting process) and the magnitude of the accompanying increase in transpiration rate. A similar remark applies to the increase in stomatal aperture. There is, however, a distinct relationship between the temperature during the experiment and the length of the interval between the commencement of wilting and the occurrence of the maximum transpiration rate; this interval may be briefly termed the 'preliminary acceleration period'. Table II shows that the length of this period varies approximately inversely as the temperature. Experiments 39, 43, and 46 are aberrant, but with the data available two of these discrepancies can be accounted for. The relative humidity of the air in the greenhouse in which the experiments were carried out generally varied inversely with the temperature. In Experiment 39, however, the degree of saturation was unusually low for the temperature—70 per cent. instead of over 80 per cent. This would, of course, tend to increase the velocity of wilting. Conversely, in Expt. 43 the humidity was 79 per cent. instead of about 70 per cent., which was more usual at 16° C.,

and in this case the rate of wilting would be correspondingly slower. These circumstances would tend to displace the respective experiments from their position in Table II in the direction of the positions in which they are actually found. The aberration of Expt. 46 remains unaccounted for, but on the whole the series is consistent, and shows the close correlation between the air temperature and the duration of the preliminary acceleration period. Consideration of the stomatal aperture figures in Table II shows an exactly similar correlation existing between the air temperature during wilting and the duration of the preliminary period, with the reservation that Expt. 43 is again aberrant. This discrepancy is amenable to explanation as in the case of the transpiration results. This correlation between air temperature and the rapidity with which maximum stomatal aperture is reached has been observed and recorded in a less complete form by Laidlaw and Knight (16), and it is significant that a similar relationship has been found when studying transpiration rate. There appears to be no doubt that these maxima in the graphs of stomatal aperture and transpiration rate represent a definite stage in the physiological changes accompanying wilting. The more rapid attainment of these maxima which is associated with higher air temperatures is an indication that the plant is wilting more rapidly, and in fact, as Laidlaw and Knight suggested, the duration of the preliminary acceleration period is an indication of the rate at which the plant is wilting.

Another point which may be considered in this connexion is the relation between the preliminary acceleration period of the stomata and that of the transpiration rate. Including the figures in Table II, there are available for comparison nine experiments in which complete records of stomatal aperture and of transpiration rate were obtained. In four of these the maxima of the two graphs appeared simultaneously, in five the maximum of transpiration was reached before that of stomatal aperture, and in none did the stomatal aperture maximum appear first. It must be pointed out that all the recorded figures are averages over periods of varying lengths, and that therefore, whilst an apparent difference between the times of the occurrence of the two maxima indicates a real difference, on the other hand apparent simultaneity does not necessarily mean absolute simultaneity. In the present experiments, had there been an interval of three or four minutes between the appearance of the two maxima, the chances are about even that this interval would have been overlooked. Therefore we may accept the five cases in which the maximum of transpiration rate was reached first, but we are unable to pass judgement on the other four. These five cases provide an instance of the influence of leaf water-content on transpiration rate, irrespective of the effect of stomatal aperture. During the period between the transpiration maximum and the stomatal aperture maximum, the transpiration rate diminishes although the stomatal aperture is increasing.

This is no doubt due to the fall in leaf water-content and indicates the onset of wilting. This constitutes a special case of the well-known generalization which has now been frequently demonstrated, that in its daily course the transpiration rate reaches a maximum before the atmospheric evaporating power, owing to the check exerted by the decreasing water-content of the leaf.

Having demonstrated by this series of experiments the occurrence of a rise of transpiration rate accompanying the temporary opening of the stomata which occurs during wilting, it is necessary to consider these phenomena in relation to the rise of transpiration rate which Bakke has recorded in association with permanent wilting. It has been pointed out earlier that the interval elapsing before the appearance of the transpiration increase accompanying permanent wilting is very much longer than the interval in the present experiments, but in such a connexion time intervals are useless for comparison unless some criterion of physiological condition is available. It may be urged that in Bakke's experiments the plants were wilting slowly as the result of the gradual failure of the soil water-supply, whereas in the present investigation the velocity of wilting was much greater owing to the sudden complete cessation of the water-supply. This is true, but it is equally true that in the present case, even with the more rapid wilting, the plants never approached the stage of permanent wilting. In order to be sure of this, at the end of each experiment, when transpiration rate was falling and the stomata were closing, the stopcock of the potometer was reopened, allowing water to enter the cut stem again. In every case the shoot recovered its turgidity in the ordinary greenhouse atmosphere without recourse to increased humidity, thus demonstrating that the permanent wilting stage had not been reached. It is evident, then, that the stomatal opening and rise of transpiration rate which have been shown to accompany the initial stages of wilting cannot be associated with the rise of transpiration found by Bakke at the permanent wilting stage. This statement is not intended to rule out the possibility of the recurrence of stomatal opening at a later stage of wilting. In the course of a large number of experiments on the behaviour of stomata during wilting, the writer has never seen any indication of such recurrence, but, although leaves have frequently been allowed to wilt to extreme flaccidity, in none of the experiments has either Bakke's or Shive and Livingston's test for permanent wilting been applied.

An attempt was next made to discover whether the attainment of the maxima of stomatal aperture and transpiration rate represented a stage of the wilting process which could be associated with a definite water-content of the leaf. The relation between the time occupied in reaching these maxima and the air temperature during wilting appeared to indicate that these maxima might be found to occur when a definite quantity of water

had been lost from the leaves. Experiments were therefore carried out to determine the change of water-content of the leaves during the preliminary acceleration period. The water-content of the cells concerned in transpiration and stomatal change will depend upon the rate at which water passes to these cells from other parts of the plant. Cribbs (6) states that there is evidence that a water deficit may be due to the failure of the water-translocating system to supply water to the leaves in sufficient quantity to counterbalance the loss by evaporation. It appears that he includes in the term 'translocating system' the path of the water through the soil and into the roots, as well as the conducting system of the plant. It is self-evident that in a dynamic system such as the plant, the water-content of any particular region must be dependent upon the rate of water translocation. It is evident also that changes in the water-content of the whole plant are not necessarily indications of changes in the water-content of any particular cell. It is therefore inadvisable to use the former and impossible to use the latter for experimental determinations. As a practicable compromise the water-content of a single leaf lamina was adopted as a working basis, and this was determined in conjunction with measurements of stomatal changes, since the latter are easier to obtain and to localize than measurements of transpiration rate. In addition, change of transpiration rate is in the present instance probably almost wholly dependent upon change of stomatal aperture. At the outset, determinations were made of the water-content of leaves of all ages from plants of several species, including *Eupatorium adenophorum*, which had been used for many of the foregoing experiments. Considerable differences of water-content were found to exist, as indicated in Table III, which gives the figures for all the leaves of one shoot.

TABLE III.

Water-content of single leaves of *Eupatorium adenophorum*. 1 A and 1 B are two companion leaves from the same node. The same applies to 2 A and 2 B, &c.

Leaf No.	% water calculated on wet weight.
1 A } youngest pair	81.8 }
1 B }	82.2 }
2 A }	85.7 }
2 B }	85.5 }
3 A }	86.1 }
3 B }	86.0 }
4 A }	85.3 }
4 B }	84.9 }
5 A }	84.1 }
5 B }	83.9 }
6 A }	84.9 }
6 B }	84.3 }
7 A }	85.5 }
7 B }	85.8 }

There is a great variation in the water-content of different leaves, even if the very young leaves at the tip of the shoot are excluded, but the difference between the two companion leaves from the same node is much smaller, amounting on the average to 0.3 per cent. with a maximum in 6 A and 6 B of 0.6 per cent. Large differences in the water-content of different aged leaves were also found in the other species used, which included a *Fuchsia* species, an *Abutilon*, ivy-leaved *Pelargonium*, *Morus nigra*, and *Pleroma macrantha*. In view of the results obtained with companion leaves, trials were made with companion leaflets of pinnate leaves of a weeping ash and *Juglans regia*, and it was found that the figures obtained for these were in even closer agreement than those from companion leaves.

Having obtained information with regard to the water-content of different leaves, the procedure was as follows: A record of stomatal aperture was obtained from a leaf under ordinary conditions, and at a convenient time the leaf was detached from the plant and allowed to wilt, the stomatal record being continued. When this leaf was detached its companion leaf was also detached and its water-content determined. When the stomata of the wilted leaf had ceased their preliminary opening movement and had begun to close, the stomatal record was stopped, and the leaf was removed from the leaf-chamber, weighed, and dried in the usual manner for water-content determination. The difference between the two values thus obtained represented the change of water-content of the leaf in passing from a condition of turgidity to the stage of wilting marked by the attainment of maximum preliminary stomatal opening. Account must of course be taken of the limits of accuracy imposed by the variation in water-content of companion leaves, which was demonstrated by Table III. The results of a series of these experiments are given in Table IV.

TABLE IV.

Showing change of water-content of a wilting leaf during the preliminary acceleration period.

1 and 2, *Fuchsia* species. 6, 7, 8, *Eupatorium adenophorum*.

10, 11, *Abutilon* species.

Water content of leaf.

<i>Experiment No.</i>	<i>Turgid.</i>	<i>When stomata had ceased to open.</i>	<i>Difference.</i>
1	88.9	88.7	-0.2
2	88.1	87.6	-0.5
6	87.2	87.4	+0.2
7	87.2	85.6	-1.6
8	86.8	86.7	-0.1
10	78.4	76.8	-1.6
11	79.9	78.8	-1.1

In no case was a decrease in water-content of more than 1.6 per cent. found, and it is possible that as much as 0.6 per cent. of this was the original difference between the two companion leaves. The average decrease must be very small, and with the method used it is impossible to determine it with accuracy. The result was rather surprising, since the leaves showing this small decrease in water-content were definitely flaccid. To supplement these results a series of leaves were removed from the plant and allowed to wilt for ten, twenty, and thirty minutes respectively before weighing for water-content determinations. Observations were made of the appearance of the leaves at the time of weighing and determinations were carried out on companion leaves at the same time, as indicated in Table V.

TABLE V.

<i>Water-content of turgid leaf.</i>	<i>Duration of wilting period.</i>	<i>Water-content at end of period.</i>	<i>Decrease in water- content.</i>	<i>Appearance of leaf.</i>
84.7	10 min.	83.9	0.8	Leaf distinctly flabby.
86.0	20 "	85.4	0.6	Limp, lustre lost.
84.7	30 "	83.9	0.8	Limp.

These figures demonstrate that extreme flaccidity results from a decrease of approximately 1 per cent. in leaf water-content. In fact it is easier to detect the early stages of wilting by direct observation than by determination of decrease of water-content. It must be emphasized that the foregoing series of experiments does not actually demonstrate that the stomatal opening which occurs during wilting is brought about by a loss of 1 per cent. of the water in any particular portion of the leaf—for example, the epidermal cells. The leaf has been treated as a whole in the water-content determinations, and it is conceivable that, although the mean decrease is only 1 per cent., the water concerned in wilting, i.e. that contained in the cell sap, may have decreased by a much larger amount. At the same time it is remarkable that a mean decrease of this magnitude should result in such a far-reaching effect as the production of the extreme flaccidity observed.

Interpretation of the phenomenon in terms of single cells results in the rather surprising conclusion that the cell wall is only very slightly distended in the normal turgid state of the cell. The loss of small quantities of water from a cell with an elastic cell wall would simply result in a slight general contraction of the whole structure without any such loss of turgidity as is indicated by the collapse of the leaf. It may of course be possible to distend the cell walls of an already turgid plant by restricting transpiration whilst facilitating water absorption, but the foregoing experiments dealt with typical mesophytes in normal environment, and it is under these conditions that the conclusions are intended to apply. Since visible flaccidity is brought about by such small changes of water-content it is evident that the water

balance in the plant must be an extremely delicate one. As water is lost by transpiration it must be replaced by translocation of water from the petiole or the stem, and this simultaneous progression may even extend to root absorption, thus permitting the maintenance of almost perfect equilibrium. Otherwise even a small change of transpiration rate induced by slight alteration of the environmental conditions would immediately result in a change in the water-content; a small increase in the wind velocity, for example, would produce visible wilting in a very short time. The leaves of a few plants, e.g. *Helianthus*, the large-leaved species of *Saxifraga*, and the root crops, certainly do lose some of their turgidity on very hot days, but it is the exception rather than the rule to find the ordinary mesophytes wilted even on the days of highest atmospheric evaporating power. In the absence, therefore, of any regular occurrence of temporary wilting, it must be concluded that changes of transpiration rate are accompanied by equal compensating changes of absorption rate. Such close correlation between these two processes is more easily conceivable if part at least of the flow of water through the plant is maintained by the pull, upon continuous tensile water columns, resulting from evaporation and capillarity, than if the elevation of water is effected by directive 'vital' action of leaves or stem. (See Dixon (10), pp. 24, 25.)

Another aspect of the question of change of water-content is worthy of consideration. It appears paradoxical that whilst a change of 1 per cent. in leaf water-content may produce such profound results, yet two leaves on the same shoot, apparently quite similar as regards turgidity, may differ in water-content by as much as 4 per cent. This apparent discrepancy may be the result of structural differences—for example, difference of thickness of cell wall. A leaf is a composite structure made up of many different types of tissue, doubtless differing in the percentage of water which they contain. It is evident that a difference in the proportion of the various tissues in two leaves would result in a different percentage water-content of the leaf as a whole.

The variation of leaf water-content during the day and night is rather a different problem and needs special consideration in relation to the results already recorded by other workers. Clark (5) has investigated changes of water-content of leaves of trees, and found that in addition to a seasonal change from a maximum in spring to a minimum at leaf fall there also occurred considerable variations through the day. It was nevertheless found impossible to determine the time of day at which the maximum or minimum water-content was reached, on account of the variation in the results.

The seasonal change of water-content is attributed by Clark to seasonal structural changes similar to those postulated above, but no explanation is advanced to account for the large diurnal changes which were recorded. The difference in leaf water-content determined at different times of the

day amounted in one case to more than 20 per cent. (76.6 per cent. at 10 a.m., 54.7 per cent. at 3 p.m.). Assuming that the dry matter of the leaf remained approximately the same, this means that of the original water in the leaf about 65 per cent. has been lost. This excessive loss of water is very surprising in view of the experiments previously recorded in the present paper, especially as Clark makes no mention of wilting. It is possible, however, that the leaves used in Clark's experiments for water-content determinations at different times of the day were never really comparable at all, but that their respective water-contents differed very considerably at the outset. The present paper has shown that considerable differences may exist even between apparently similar leaves, and Clark has recorded no attempt to determine whether such initial differences existed in the plants which she used. The above interpretation of the enormous differences found by Clark receives additional support from the fact that she found it impossible to associate these variations in water-content with changes of temperature or humidity, or with the changes of evaporating power of the air which occur at different times of the day.

Clark also states as a general conclusion that water-content is independent of transpiration, temperature, and humidity. A study of Clark's results shows that this statement means that water-content *varies* independently of transpiration, temperature, and humidity. The experimental evidence offered in previous sections of the present paper indicates that, on the contrary, the leaf water-content must remain constant within a very narrow range throughout the day. The flow of water into the leaf appears to be nicely adjusted to counterbalance the loss by transpiration, and the total amount of water in the leaf cells is almost unchanged. Extreme conditions, however, whether caused by excessive transpiration or by lack of soil moisture, might be expected to disturb this delicate equilibrium, at the same time producing flaccidity. In the sense, then, that water-content is almost constant and unaffected by variations of transpiration and external conditions, it may be said to be independent of these changes, but this interpretation of Clark's statements is quite at variance with her results, and is not the one which she accepts.

The work of Livingston and Brown (17) and of Shreve (19) is relevant to the present work. Investigations carried out by these workers showed that under desert conditions there was a definite diurnal cycle of changes in the water-content of the leaf. A minimum quantity of water was found to be present during the period of high evaporation rate, whilst the maximum water-content was reached at some time during the night when transpiration was low. This would appear to be the natural result of a limited water-supply, in consequence of which the passage of water into the leaves is not sufficiently rapid during the daytime to replace the water lost by transpiration. The influence of these conditions upon transpiration rate has been

discussed at length elsewhere by various writers and need not be further considered here, but it is necessary to refer to the work of Livingston and Brown, which has a bearing on the phenomena at present under consideration. In their carefully controlled experiments, the difference between the maximum and the minimum leaf water-content was never more than 8 per cent., and in one case, *Physalis*, only 1 per cent., with an average for all recorded cases of 5 per cent. This figure, obtained under extreme atmospheric conditions, is widely different from that obtained by Clark, but is probably a much more accurate estimate, as the experiments recorded below tend to show. In view of the very small decrease in leaf water-content which will produce flaccidity, it is evident that if during the normal daily cycle the leaves of the plants concerned exhibit fluctuations of water-content such as those recorded for the desert habitat, the plants must daily suffer a considerable degree of wilting. This is not the case; on the contrary, the plants under consideration do not wilt to an extent appreciable by observation except in a high wind or as the result of deficient soil moisture. Consequently it was thought desirable to apply the method of Livingston and Brown to the species used in the present work in order to determine the extent of the diurnal changes of leaf water-content. The plants were living in a cool greenhouse or under temperate outdoor conditions, not approximating to those of the desert habitat of the plants of Livingston and Brown. Experiments were carried out with ash, apple, and quince out of doors and with *Eupatorium*, *Peristrophe*, and *Cycas* in a greenhouse. In the case of apple and quince large numbers of leaves were taken and the probable errors were calculated. In dealing with the other species companion leaves or leaflets were compared in the manner indicated in experiments recorded above.

The period of maximum water-content of the leaves used by Livingston and Brown occurred during the morning hours, whilst the minimum was reached during the afternoon. With this result in view determinations were made in the present experiments at two different times of the day; for example, at 8 a.m. when the atmospheric evaporating power was low and the loss by transpiration small, and again at 2 p.m. after a period of rapid transpiration. Table VI gives the result of an experiment using pairs of leaves of *Eupatorium adenophorum*.

It has previously been emphasized in this paper that in experiments on the water-content of pairs of leaves it must be recognized that the accuracy is limited by the extent of the initial difference between the water-contents of the two companion leaves. The last two columns of the table show that the evaporating power of the air, as determined by a Livingston porous cup atmometer, was at its highest during the morning, between the 8.30 a.m. and the 1 p.m. water-content determinations, whilst during the period between the 1.15 p.m. and the 5.15 p.m. determinations it had fallen to a quarter of its morning value.

TABLE VI.

Variation of leaf water-content at different times of the day. *Eupatorium adenophorum*. Oct. 15, 1919.

8.30 a.m.	1 p.m.	Change.	1.15 p.m.	5.15 p.m.	Change.	Water loss from spherical cup atmometer.	
%	%		%	%		Period.	Grm. per hour.
80.4	79.1	-1.3	82.4	82.5	+0.1	8.30 }	0.68
84.2	83.3	-0.9	79.1	79.8	+0.7	9.30 }	
84.8	83.8	-1.0	80.7	81.7	+1.0	9.30 }	4.72
84.1	83.2	-0.9	82.6	82.2	-0.4	12.45 }	
83.4	82.7	-0.7	79.7	79.9	+0.2	12.45 }	4.56
82.0	81.0	-1.0	81.5	81.6	+0.1	2.0 }	
83.5	82.7	-0.8	81.8	82.7	+0.9	2.0 }	3.09
82.3	81.9	-0.4	81.4	82.4	+1.0	3.50 }	
83.0	83.3	+0.3	83.4	84.0	+0.6	3.50 }	1.11
82.0	81.7	-0.3	81.1	82.0	+0.9	5.30 }	
82.5	83.8	+1.3	81.4	81.1	-0.3		
81.6	81.7	+0.1	82.5	82.9	+0.4		
Mean :	{ -0.5 ± 0.1		Mean :	{ +0.4 ± 0.1			
Temperature			{ Max. 14.7° C. 12.30 p.m. Min. 6.4° C. 8.30 a.m.				
Relative humidity			{ Max. 86% 5.0 p.m. Min. 57% 11.30 a.m.				

Twelve pairs of leaves were dealt with over each period, and between 8.30 a.m. and 1 p.m. the largest decrease in water-content was 1.3 per cent. During this same period one pair of leaves showed an *increase* of 1.3 per cent., but it is of course probable that this was due to an initial difference in water-content between the two companion leaves. The average decrease of the twelve pairs was 0.5 per cent. with a probable error of 0.1 per cent. for this average. Three pairs showed an increase in water-content.

The second portion of the experiment covered the afternoon hours when the atmospheric evaporating power, and doubtless also transpiration rate, were rapidly decreasing. Samples taken at 1.15 p.m. and 5.15 p.m. showed an average increase in water-content of 0.4 per cent., probable error 0.1 per cent. Two samples showed a decrease.

Similar experiments have been carried out with a number of species and a *résumé* of the results is given in Table VII.

Since it was evident that if the water-content of leaves decreased by more than about 1 per cent. flaccidity must occur, it was expected that no large diurnal changes would be found. Accordingly it was not surprising that in this series of determinations there is no indication of any diurnal change of leaf water-content of a magnitude corresponding to those found by Livingston and Brown, or even approaching those recorded by Clark.

TABLE VII.

Summary of experiments on various species to determine changes of water-content through the day.

Experiment No.	Species.	Time of first determination.	Time of second determination.	Change in water-content.			No. of comparisons.
				Average.	Max.	Min.	
83	<i>Eupatorium adenophorum</i>	8.0 a.m.	2.0 p.m.	-0.25	-1.8	+0.7	6
86	"	9.0 "	2.0 "	-0.6	-1.1	—	6
88	"	8.30 "	1.0 "	-0.5	-1.3	+1.3	12
88	"	1.15 p.m.	5.15 "	+0.4	+1.0	-0.4	12
91	<i>Peristrophe speciosa</i>	8.30 a.m.	1.15 "	-0.2	-0.6	+0.2	8
93	<i>Cycas circinalis</i>	8.45 "	1.30 "	-0.1	-0.4	+0.6	6
94	<i>Peristrophe speciosa</i>	11.30 "	4.15 "	+0.1	—	—	6
109	Quince	10.0 "	3.30 "	+0.4 ± 0.1	+0.6	+0.1	6
110	Apple	3.30 p.m.	9.30 a.m.	-0.1	—	—	40
107	<i>Fraxinus</i>	10.0 a.m.	3.30 p.m.	-4.4 ¹	-7.0	-3.0	8
108	"	9.0 "	4.0 "	-1.4 ¹	-2.9	+1.3	20

The writer does not offer any complete explanation of these differences, but suggests that the wide divergence between the habitats of the plants used by Livingston and Brown and of those in the present investigation may account for the difference in behaviour. It is evident that a plant which can suffer a decrease in water-content of 8 per cent. without becoming flaccid is more fitted to flourish in the extreme conditions of a desert habitat than one which loses its turgidity if its leaf water falls from 85 per cent. to 83 per cent.

SUMMARY.

It has been demonstrated by simultaneous observations that an increase of transpiration rate accompanies the stomatal opening which occurs during the early stages of wilting.

There is no correlation between the temperature of the air in which wilting occurs (i.e. presumably the rate of wilting) and the magnitude of the accompanying increase in transpiration rate or the magnitude of the increase of stomatal aperture.

The time elapsing between the commencement of wilting and the attainment of the maxima of transpiration rate and stomatal aperture is dependent on the rate of wilting. Thus the attainment of these maxima represents definite stages of the wilting process.

These stages are reached very early in the process, before the water-content of the wilting leaf has decreased more than about 1 per cent.

¹ These shoots were intentionally allowed to wilt between the early and the later determinations to obtain an indication of the magnitude of the water loss corresponding with visible wilting. In the notes of these experiments the leaves in No. 107 are described as 'badly wilted', and those in No. 108 as 'slightly wilted'.

The commencement of wilting may be inferred from the flaccid condition of the leaves before it is possible to determine experimentally a definite decrease in leaf water-content.

The loss of leaf turgidity following such a small decrease in leaf water-content indicates that the wall of a normally turgid cell is only slightly distended.

The water-contents of leaves apparently similar may differ by as much as 2 per cent. in *Eupatorium adenophorum*, and more in other species.

The diurnal change in leaf water-content in south-eastern England amounts to less than 2 per cent., much less than the maximum variation (8 per cent.) previously recorded for desert habitats.

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Sequoia Couttsiae, Heer, at Hordle, Hants: A Study of the Characters which serve to distinguish Sequoia from Athrotaxis.

BY

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With five Figures in the Text.

IN a note published recently¹ I drew attention to the fact that the Conifer remains of the Lower Headon Beds of Hordle (Hordwell), Hants, which Mr. Starkie Gardner had referred to *Athrotaxis*,² must now be placed in the genus *Sequoia*.

This conclusion was based on the study of leaves, cones, and seeds, the relationship of these organs being proved by the discovery of one cone attached to its twig, and of another still bearing seeds. The material examined was collected from the Hordle 'Leaf-Bed',³ where it is very abundant.

A comparison of the Hordle and Bovey Tracey specimens has shown that, though in error as to the generic name, Mr. Starkie Gardner was right in classing the Bovey and Hordle fossils as the same species, for the two plants are identical in character. Hence the excellent specific description of Heer⁴ renders superfluous further reference to the macroscopic characters, except in regard to certain points not mentioned by him. These will be dealt with as occasion requires in treating of the different plant organs.

To the earlier descriptions of the twigs there is nothing new to add, their condition being such as to render section-cutting unprofitable labour.

Microscopic study of the leaves shows that stomata occur on both

¹ Ann. Bot., vol. xxxv, No. cxxxix, July 1921.

² Starkie Gardner: Monograph of Palaeontographical Soc. The Eocene Flora, vol. ii, 1883-6, p. 90, Pl. X, Figs. 6-9.

³ Bed 10 of Tawney and Keeping: Quart. Journ. Geol. Soc., vol. xxxiv (1883), p. 566. On the Section at Hordwell Cliffs.

⁴ Heer: Phil. Trans. Roy. Soc., Part III, 1862. On the Lignite Formation of Bovey Tracey, Devonshire, p. 33, Plates VIII, IX, X.

surfaces; on the lower they are irregularly scattered from apex to base, but are few in number, or absent, around the margin and along the median dorsal angle. On the upper they are very abundant, occurring more or less unevenly on either side of the median line, both on the decurrent and falcate parts of the leaf, but they are absent from the median line itself and from the thick margin (Fig. 1, *b*). Nevertheless, despite the general differentiation into stomata-bearing and non-stomata-bearing regions, the limiting line between the two is ill-defined and sinuous. Though the general distribution of the stomata can readily be seen, the actual details of cell structure can only be distinguished occasionally. Each stoma is ringed by about four narrow, parallel-sided cells (Fig. 2, *b*), and usually three or more stomata occur in a row placed end to end, while the

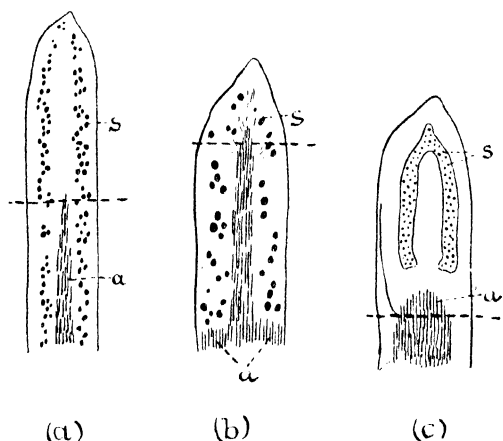


FIG. 1. Distribution of stomata on the upper surface of leaves. (a), *Sequoia gigantea*; (b), *S. Coultisiae*; (c), *Athrotaxis laxifolia*; s = stomata. Above dotted line = falcate part of leaf. Below dotted line = decurrent part of leaf. a = attached area.

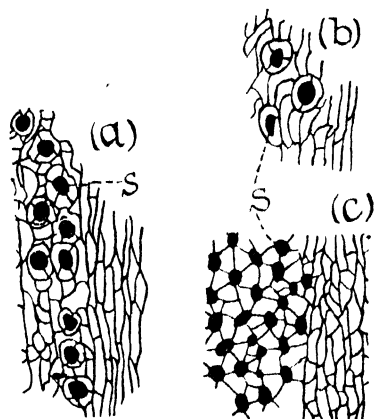


FIG. 2. Detail of stomata and non-stomata-bearing cuticle. (a), *S. gigantea*; (b), *S. Coultisiae*; (c), *A. laxifolia*; s = stomata.

cells of the interspaces have curved outlines. In areas where stomata are absent the cuticle cells are elongate parallel to the length of the leaf.

A comparison of the fossil with recent *Sequoia* and *Athrotaxis* shows that it is related to the former (compare Fig. 1, a, b, c, and Fig. 2, a, b, c), for in *Athrotaxis* the stomata on the lower surface of the leaf occur only where the decurrent base merges into the falcate portion (compare description of *Sequoia* above), while on the upper surface they are confined to the falcate region, where they occupy a clear-cut, inverted-V-shaped tract shown in Fig. 1, c. There is no aligned arrangement; instead, stomata are evenly and thickly scattered over the whole V-shaped area; the cells between them are triangular and are radially arranged (Fig. 2, c). The evidence from the leaves is, then, fairly conclusive that the fossil belongs not to *Athrotaxis* but to *Sequoia*.

In Heer's account of the cones of *S. Couttsiae*¹ the following features, which have a generic significance, have been overlooked. The seminiferous scale and bract are almost completely fused to form a single structure—the cone-scale. In each cone-scale there are two regions, the stalk and the escutcheon; in the latter only do traces of the original double structure of the scale remain, but there a small mucro and associated transverse ridge seem to represent the apex of the bract and the line of fusion respectively. This is a character of recent *Sequoias*, though in these the complete ridge is only seen in the basal scales of *S. sempervirens*; in all other scales of both species the mucro is sunk in a groove and the ridge is apparent only at the edge.

The surface of the escutcheon is always ornamented with radial wrinkles. Its form may be quadrilateral, pentagonal, hexagonal, or irregular, and its margin is always clearly defined (Figs. 3, *a*, *b*, and 4, *a*, *c*); it is set at a marked angle to the stalk, there is never a gradual passage from one to the other. The stalk occasionally meets the escutcheon symmetrically at its centre and at a right angle; more frequently, it meets it asymmetrically, towards its lower margin and at an obtuse angle; this angle is greatest in the lowest scales, where it may be about 150°. Thus there are two extreme forms of scale—peltate and imbricate—with a variety of intermediate forms.

In the recent *S. sempervirens* both these types are present; the imbricate character is, however, confined to a few basal scales (Fig. 3, *c*). In *S. gigantea* it is completely lost, and only peltate scales remain. Apparently it was the presence in the fossil of imbricate scales which led Mr. S. Gardner to identify it as *Athrotaxis*, for he wrote that an uncrushed specimen 'revealed the fact that the scales of the cone are overlapping or imbricate as in *Athrotaxis*, and not at right angles to the axis as in *Sequoia*'.² As the new evidence from *S. sempervirens* shows that imbricate scales can occur, their presence in the fossil is not sufficient cause for placing it in *Athrotaxis* rather than in *Sequoia* when all other evidence points to the latter genus, as will be shown.

The cone-scales of *Athrotaxis* differ radically from those of the fossils and of the recent *Sequoia*. In *Athrotaxis* bract and seminiferous scale retain their identity to a marked degree. The thin, horny bract projects as a pointed tongue beyond the woody, seminiferous scale; hence the lower side of the cone-scale shows a continuous surface of bract from attachment to apex. There is no differentiation into stalk and escutcheon, and no ornamentation of the exposed surface. The seminiferous scale has a thickened, involute margin (Figs. 3, *d*, and 4, *b*, *d*).

Further, while *Sequoia* and the fossil agree, *Sequoia* and *Athrotaxis* differ widely in respect of the number and mode of attachment of the seeds

¹ Loc. cit.

² Loc. cit.

on each scale. In the fossil and in *Sequoia* the attachment is at a third of the length of the scale from its distal margin, in *Athrotaxis* the attachment scars are found immediately adjacent to the involute margin (Fig. 4, *b*); and again, in the fossil five and six seeds have been counted on a scale, while recent *Sequoia* scales each bear from five to nine; but in *Athrotaxis* the maximum is five, and the limits of variation are from three to five.

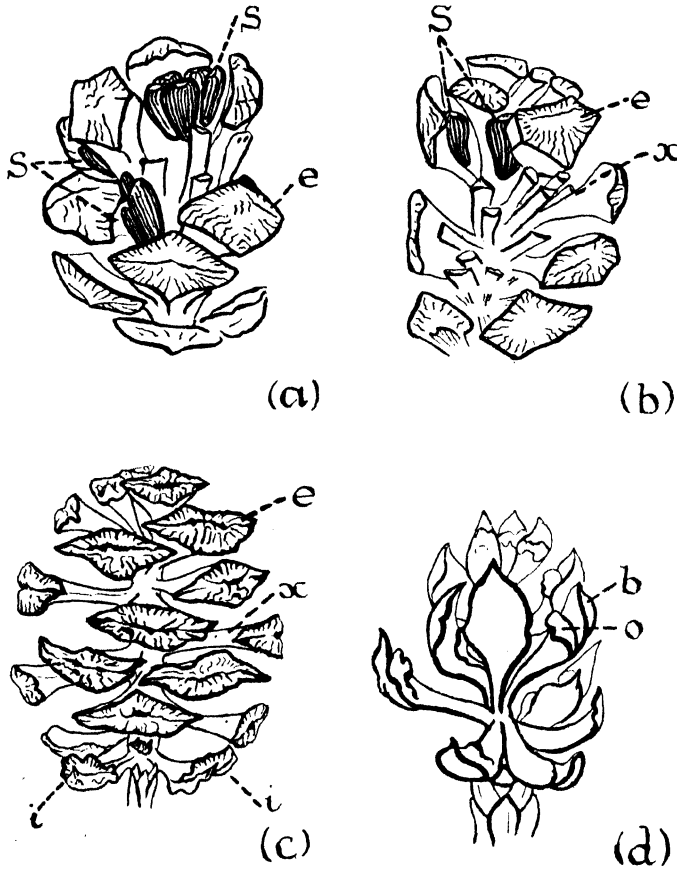


FIG. 3. Cones. (a) and (b), two aspects of a cone of *S. Couttsiae*; (c), *S. sempervirens*; (d), *Athrotaxis laxifolia*. *s*, seeds; *i*, imbricate scales; *o*, seminiferous scale; *b*, bract; *e*, escutcheon; *x*, stalk. All magnified.

Once again, therefore, there is adequate reason for referring the fossil to *Sequoia*.

To the previous descriptions of the seeds of *S. Couttsiae* I would add that considerable variations of size and shape occur; the smallest specimens measured were 2.5 mm. long and 2 mm. broad, the largest 5 mm. long and 2.5 mm. broad. The shape varies from a broad oval to a very narrow one; sometimes the apex is provided with a mucro, and though a curved embryo

is common, straight ones are found too, both in the Bovey and in the Hordle material. The embryo in the fossil is long and narrow, and the wing is thick and horny in texture. The testa is covered with close longitudinal striae whose direction of curvature conforms over the embryo

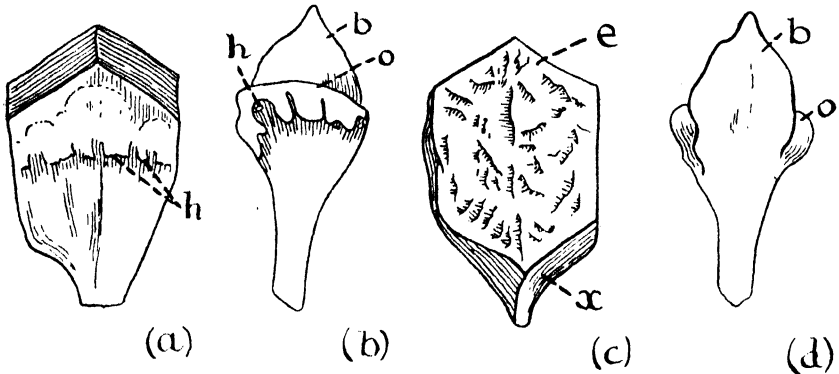


FIG. 4. Cone-scales. (a), *S. Coultsiae*, upper surface. (c), Do., lower surface. (b), *Athrotaxis luxifolia*, upper surface. (d), Do., lower surface. e, escutcheon; x, stalk; b, bract; o, seminitorous scale; h, attachment of seed. All magnified.

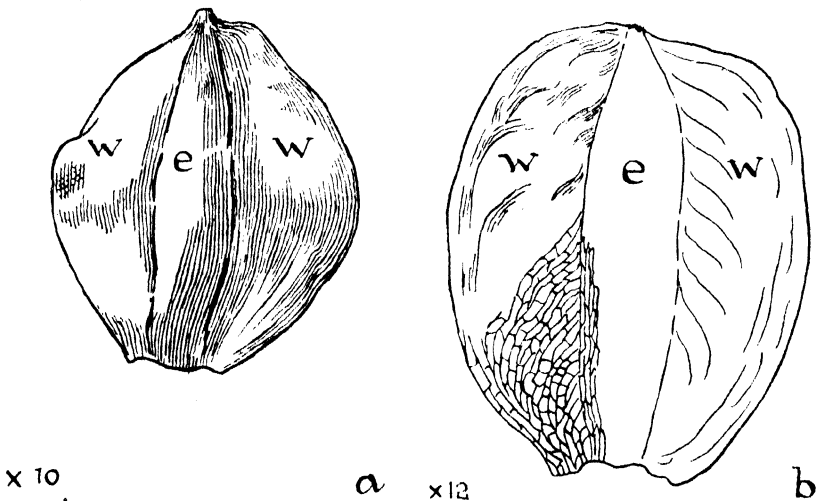


FIG. 5. Seeds. (a), *S. Coultsiae*; (b), *Athrotaxis selaginoides*. e, embryo; w, wing.

to the curvature of the embryo, over the wing to the curvature of the wing (Fig. 5, a). In the hollows between the striae pits occur in longitudinal rows; they are most conspicuous in worn specimens. The likeness which the fossils bear to recent *Sequoia* seeds, more especially to *S. sempervirens*, is very striking.

In *Athrotaxis* the seeds, instead of being horny and tough all over

have a membranous, puckered wing, whose surface is formed of large, inflated, oblong or polygonal cells. Only near the margin are their longer axes parallel to the lateral edge of the wing, elsewhere they are inclined obliquely to it; thus they are arranged in lines which sweep transversely across the wing, meeting the edge of the embryo at an angle of about 50° (Fig. 5, *b*).

The work of Mr. and Mrs. Clement Reid has shown that seed character is an admirable generic guide, and in the present instance the seed characters are so distinctive that, had seeds alone been available for study, I should unhesitatingly have assigned the fossil to *Sequoia*; but when, as in this case, evidence from other organs all points in one direction, the case for *Sequoia* appears to be indisputable.

This work was made possible by a grant from the Scientific and Industrial Research Department.

With great pleasure I acknowledge my debt to Mrs. Reid, whose experience and collection of seeds have been freely placed at my disposal, and who has also given valuable help and criticism.

I would also thank the Director of the Royal Gardens, Kew, for kindly lending herbarium sheets of *Athrotaxis*.

The Soils of Blakeney Point : A Study of Soil Reaction and Succession in Relation to the Plant Covering.¹

BY

E. J. SALISBURY.

With Plate XV and five Figures in the Text.

1. INTRODUCTORY.

THE work connected with this investigation was in part carried out in the Field Laboratory at Blakeney Point, where the bulk of the hydrogen-ion estimations were made upon the spot.

The purpose in view was to see to what extent the phases in the development of the maritime plant associations could be related to variations in real acidity, and how far this in turn was correlated with the leaching out of carbonates and the organic content of the soil. In elucidating the answers to these questions the problem of the cause or causes of soil acidity is necessarily involved.

The striking and easily recognized phases of the maritime succession render this type of plant formation peculiarly suited to a study of the accompanying soil changes. It is highly probable that a similar edaphic succession to that here demonstrated characterizes other inland plant formations, and the results here presented form a striking confirmation, in a totally different type of habitat, of the edaphic succession already studied by the writer in woodland communities (*Journal of Ecology*, vol. ix, pp. 220-40, 1922).

But whereas the phases in woodland successions are often of a secular character and must in most cases be inferred from collateral evidence, the successions in coastal formations such as sand dunes, shingle beaches, and salt marshes are sufficiently rapid to present successive phases in one and the same area.

In this respect Blakeney Point offers exceptional advantages, though the final stages in the dune series are lacking owing to their removal by wind action.

¹ Blakeney Point Publication, No. 20.

The writer gladly takes this opportunity of expressing his indebtedness to Professor F. W. Oliver for having asked him to undertake a soil survey of this area. Thanks are also due to Dr. P. Haas for the preparation of standard buffer solutions, to Dr. Brady for electrometrical checks, to Lieut. G. N. Oliver for the preparation of Text-fig. 1, and to the following for assistance in the collection of soil samples and in the hydrogen-ion determinations: Miss V. Anderson, Miss S. Hurwitz, Miss E. Tyler, and Mr. L. Cole.

Except where otherwise stated the samples were taken to a uniform depth of 4 in., and 10 grm. of the undried soil were stirred up with 50 c.c. of water neutral to brown-thymol blue. In practice it was found that equilibrium was attained in half an hour, after which the extract was filtered and, after discarding the first filtrate, the hydrogen-ion concentration was determined colorimetrically by the aid of standardized buffer solutions and the usual indicators.

No detectable difference in the results was obtained by using twice as much soil, by lengthening the period of extraction, or by adding the indicator to centrifugalized samples. One may therefore assume that the varying water-content of the samples did not introduce any error into the results, nor are these vitiated by filtration.

The carbonate content was determined by means of a Collins calcimeter, applying the usual corrections, and the results are expressed as calcium carbonate in 100 grm. of soil dried at 100° C.

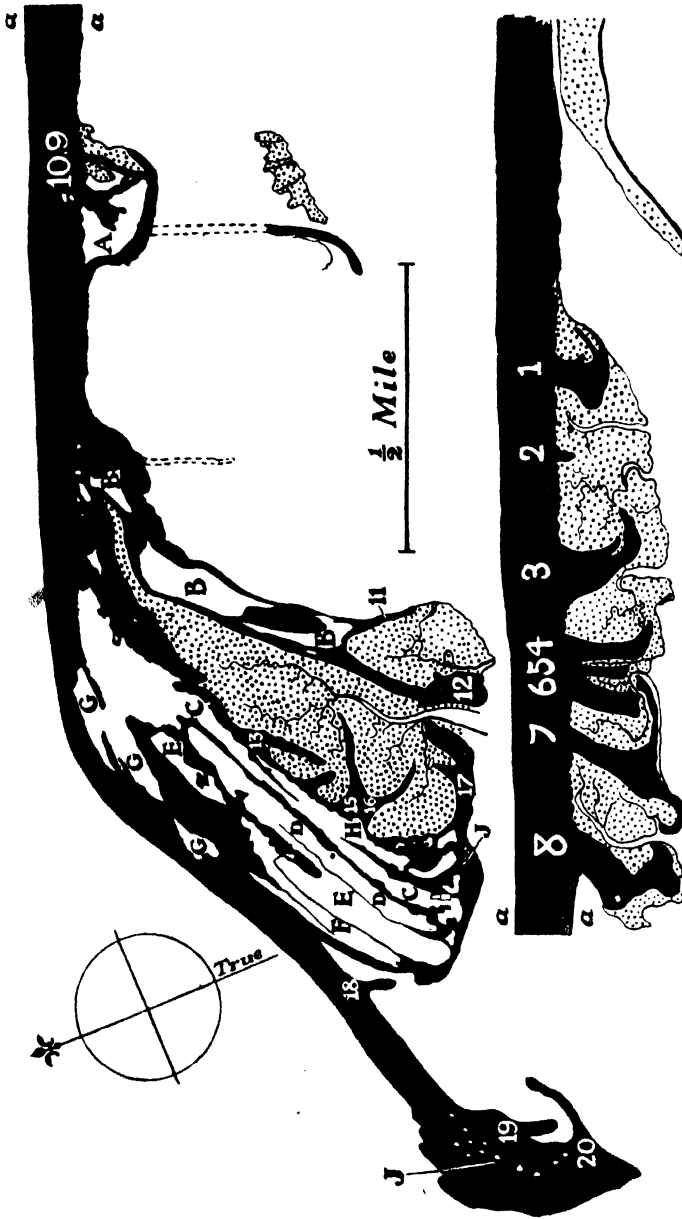
The general topography of the area and its vegetation have already been dealt with elsewhere (cf. Oliver and Salisbury, Trans. Norfolk and Norwich Nat. Soc., vol. ix, 1913), but for convenience we shall here summarize the chief features, at the same time indicating the distribution of the soil samples taken.

The salient features of the area are shown on the map (Text-fig. 1), where the sequence of numbers and letters shows the dunes and lateral shingle beaches of successive age.

A. THE DUNE SYSTEM.

The earliest phase in dune development at Blakeney Point is usually marked by the collection of sand around a young plant of *Psamma arenaria*. As the dune thus initiated accretes sand the marram grass becomes covered and is thereby stimulated to renewed growth—in this way constituting a skeletal system which enlarges *pari passu* with the growing sand-hills and permeates the entire structure of the adult dune.

Of this skeletal system the ultimate ramifications bear the green leaves at the surface, whilst the older parts, formed when the dune was young, exhibit progressive decay till in the actual heart of a large dune only disintegrated fragments may remain.



TEXT-FIG. 1. Sketch-map of Blakeney Point on a scale of 3 inches to 1 mile (1 : 21120). The lower section (marrams—clay beach) fits at *a, a*, on to the right-hand end of the upper section. *Single beaches*, where evident, are marked in black, the laterals (or 'hooks') being numbered in their presumed developmental sequence. Thus 1 is the oldest and 20 the youngest. *Dunes* are white, successive systems being lettered; *A* (the Hood) being the oldest; *B*, remnants of the 'Long Hills'; *E*, the main ridge of the headland; *J*, the newest embryo dunes. The fringing dunes *H, I, J, K, L, M, N, O, P, Q, R, S, T, U, V, W, X, Y, Z*, represent a SE. extension of the headland series from which they are subsequent derivations, especially due to sand mobility in strong NW. winds. In time, though they overlap series of the other systems, they have undergone marked accretion since the *G* system arose. *Salt marshes* are dotted. The youngest marsh to establish (since 1910) is the detached area $\frac{1}{2}$ mile south of the Hood (*A*). (Adapted by E. J. S. from air survey photographs (1921) and drawn by G. N. O.).

Thus *Psamma arenaria* plays two important rôles. On the one hand, it helps to stabilize the sand mass against wind action, and on the other adds colloidal material by which the water-content is increased and the necessary medium provided for bacterial activity.

In the outermost dunes on the sea face (cf. G, Text-fig. 1) *Psamma arenaria* is the only important species, but on the new spit (J, near 19 and 20) of shingle *Triticum junceum* also forms embryo dunes.

These early stages are represented by the series of samples G 01–G 029 and J 030–J 036. The very small new dunes actually form several groups on the sea face, and such also occur on the new spit (samples J 032–J 036) and close to the Lifeboat House on the shore of the estuary (samples J 030–J 031 and J 022–J 024). For convenience these, together with the rather older stages represented by the series of samples F 01–F 021, may be termed collectively the young dunes.

The next phase in the succession is represented by the samples E 1–E 31 from the main dune ridge (E, Text-fig. 1), where there is complete coalescence between the original units to form the highest ridge of the series. *Psamma arenaria*, though still the dominant species, is accompanied by *Festuca arenaria* and ephemerals such as *Myosotis collina*, *Phleum arenarium*, *Stellaria boreana*, *Cerastium semidecandrum*, &c.; *Senecio jacobaea* is also of common occurrence.

This phase as a whole may be conveniently termed the 'main ridge' and represents an advance in stability and organic content. It furnishes the culmination of the 'yellow dune' phase with which the marram grass is particularly associated.

Still passing landwards we come to two more ridges (D and C, Text-fig. 1) parallel to the preceding system but comparatively low in height, due to the combined effects of 'settling', loss by erosion, and greatly diminished accretion. The profile no longer presents a narrow ridge, but a broad, more or less flat top, and, associated with the changed conditions, *Psamma* is much less vigorous, whilst mosses and lichens, especially *Cladonia*, mark the advent of the 'grey dune' phase and from their abundance constitute an effective protection against wind action. Here too are encountered the highly efficient sand binders *Carex arenaria* and *Convolvulus soldanella*, which, however, attain their greatest vigour where the sand has again been rendered mobile by undercutting. This type is represented by the series of samples D 1–D 45 from the Laboratory ridge, and the series C 20–C 47 from the older and more stabilized ridge on its landward side. Two still older phases are represented respectively by the Long Hills (B, Text-fig. 1, samples B 1–B 22), where alone on the area *Polypodium vulgare* occurs, and the Hood (A, Text-fig. 1, samples A 1–A 16).

Although the Hood is almost certainly the oldest dune mass on the area its comparatively small size and its isolated and exposed position

favour accretion of sand, so that, with the exception of the depression in its centre, the soil is more mobile than might be expected, whilst the character of some of the samples is scarcely commensurate with its age.

In some dune areas all transitions up to and including heath are met with, though not present on Blakeney Point. Samples from the neighbouring system of Holkham indicate, however, that the edaphic sequence which accompanies increase of age on our area are still further accentuated in the final phrases of the succession.

The carbonate content of the dune soils (cf. Table I, Appendix). Examination of the drift line reveals at once the large proportion of shell fragments which frequently accompany the vegetable remains. After removal of the loose drift, the underlying sand is found to contain a high proportion of carbonates, sometimes over four per cent. by weight (cf. Sample X. 37, Table XI). The drift line is the especial home of *Salsola kali* and *Cakile maritima*, and the following estimations show (Table II) that the sand is here well supplied both with organic material and carbonates.

TABLE II. *Drift Line.*

Sample.	Location.	Depth.	p. H.	Organic content.	Total carbonates.
		in.		%	%
Transect across D. line	a New spit with <i>S. kali</i>	0-2	7.2	0.38	0.37
	a' " " "	4-8	7.2	0.44	0.65
	b Just above actual D. line	0-4	7.1	0.55	0.95
	c Just below " "	0-4	7.2	0.60	1.06
	d Sea front drift line with <i>Cakile maritima</i>	0-4	7.2-7.3	—	—
	e Sea front drift line	0-4	7.0	0.57	0.93

For the carbonate total, both here and on the dune soils generally, calcium carbonate is almost entirely responsible, and the figures given for the carbonate content have been calculated as CaCO_3 .

Various marine mollusca are the main source of supply on the sea face, whilst on the landward side *Paludetrina stagnalis* is probably the most important. This species makes up in numbers what it lacks in size, and the shells of dead individuals often accumulate along the drift line near the Hood to a depth of as much as nine inches and in a zone more than a foot broad. Since 900 shells of *P. stagnalis* occupy only 5 cubic centimetres, a square foot of such a drift line would represent some *two million shells*, which gives one a glimpse of the stupendous prolificacy of these molluscs and the vast numbers involved in their mortality. The shells as found in the drift contain over 97 per cent. of calcium carbonate, and hence constitute a most important source of supply.

The embryo dunes. Passing landwards from the drift line one comes to the small dunes which have arisen on the sea face and near the Life-

boat House during the period since this area has been under close observation, and are therefore known to vary between 3–8 years in age.

Examination of the carbonate content of these shows, like the drift line, a fairly high percentage, ranging from 0.28 per cent. to 0.61 per cent. by weight and averaging 0.425 per cent. To appreciate the significance of these figures it must be borne in mind that dune soils are much heavier, bulk for bulk, than ordinary soils. If, for instance, we wished to compare these data with those of woodland soil on clayey loam we should have to add about 60 per cent. to the figures for the embryo dunes.

In one cubic decimetre of an embryo dune the average carbonate content is very nearly 6.1 grm.

So long as any dune exhibits accretion each new layer of sand, consisting of particles brought in from the exposed sand of the sea-shore or the estuary, will contain its quota of shell fragments. So that, assuming growth to be fairly continuous, the embryo dune will exhibit, within broad limits, a certain homogeneity of carbonate content, in striking contrast to the diminishing content found in natural soils of ancient origin in which leaching has in course of time established a vertical gradient. (Cf. Salisbury, 'Stratification and Hydrogen-ion Concentration of the Soil in relation to Leaching', *Journal of Ecology*, loc. cit., 1922.)

Since sand grains and shell fragments have very different specific gravities (sand grains 1.42, shell fragments *c.* 2.7–2.9) the proportion of calcium in the layers deposited during any given wind storm will clearly vary with the velocity of the wind. On the whole very high winds will tend to deposit sand having a higher calcium content than winds of low velocity. On the other hand, however, it must be recognized that the larger fragments of shells present a larger surface for an equivalent volume than the more or less isodiametric sand grains, and may therefore be more readily wind borne despite their higher specific gravity.

TABLE III. *Carbonate Content of Wind-borne Sand deposited at c. 24 ft. above Mean Sea-level.*

<i>Sample.</i>	<i>Carbonate.</i>	<i>Sample.</i>	<i>Carbonate.</i>
	%		%
1	0.31	7	0.35
2	0.35	8	0.40
3	0.44	9	0.37
4	0.32	10	0.38
5	0.50	11	0.36
6	0.45	12	0.40

Total average, 0.385 %.

Analyses of sand which has accumulated in the loft of the Lifeboat House, about 24 ft. above mean sea-level, give a fair indication of the character of sand carried by the higher velocities of wind. It will be seen

that the carbonate content, though below the average for the smallest dunes, is higher in general than that of the main dune ridge. Incidentally these data show that any accretion which may occur on the older dune phases, i.e. those farthest from the sea, will be comparatively rich rather than deficient in calcium. So that any deficiency of carbonates in the older phases must be the outcome of leaching and not of selective wind action.

In view of the foregoing considerations it is not surprising to find considerable variation in the carbonate content as between the surface and subsurface of these embryo dunes. In two instances (cf. samples 011, 012 and 020, 021) the top four inches showed a lower content, whilst in a third locality the reverse condition obtained (cf. 015, 016). Judging by the p. H. values this is true also for the area represented by 017 and 018.

Passing to the main ridge, the average carbonate content is slightly lower than that in the youngest phases, viz. 0.341 per cent. This represents about 4.9 gm. in a cubic decimetre, or a decrease of about 20 per cent. as compared with the 'embryo dunes'. What period of leaching this represents is not accurately known, but probably not less than 60-80 years.

The range noted varies between 0.15 per cent. and 0.65 per cent., the lower limit being thus considerably less, about half that of the youngest dunes, whilst the maximum is slightly higher.

Since these main ridge dunes are the highest of the whole series, they will only receive comparatively large fragments of shell during high winds. From the data already given, however (cf. Table III), it is clear that such accretion will consist of sand comparatively rich in calcium, and the occasional high values on the seaward face (E 24) and in the hollow represented by E 9 are perhaps the outcome of selective wind action on shell fragments of relatively large surface.

Comparison of the hydrogen-ion values for surface and subsurface for five locations show a higher value for the subsurface in three cases (E 14, E 15; E 18, E 19; E 20, E 21). In one case the same reaction was found (E 1, E 2), whilst in the fifth case (E 8, E 9) the surface was practically neutral and the subsurface slightly acid. Carbonates were only determined in one of these pairs, and this showed a higher content at the lower level. On the whole this then would appear to be the general tendency, and would indicate a preponderance of leaching action over accretion.

All the remaining series of samples represent dunes which for the most part have ceased to accrete any appreciable amount of fresh sand, though 'blow-outs', rabbit-burrows, &c., may effect a partial, if local, rejuvenescence.

The Laboratory ridge shows an average carbonate content of 0.155 per cent., and a range from 0.04 per cent. to 0.03 per cent. The average is equivalent to about 2 gm. per cubic decimetre.

Here then the source of additional calcium being almost cut off by the high main ridge on the seaward side, the leaching action of the carbonated rain-water has gone on practically unhindered, and the low content as compared with the main ridge is a measure of the time that has elapsed since the Laboratory ridge ceased to accrete actively. For equivalent volumes of soil the average carbonate content here is under 41 per cent. of that present in the main ridge, and only about 32 per cent. of the average for the embryo dunes.

The marked leaching action which these data indicate is also reflected by the strikingly different carbonate content which may obtain between the surface and subsurface (e. g. D 13, D 14), and the fact that the surface inch may be entirely devoid of carbonates (cf. samples C 20, C 40, C 41).

The Long Hills show the same phenomenon in a more advanced phase. The proximal end of these where they abut on the main beach is not cut off from the sea face by the main ridge which stops short of this junction. The actual distance from the sandy foreshore is here, moreover, only about 260 yds. It is in correspondence with this that the sea face at the proximal end exhibits a much higher carbonate content than the Long Hills generally, viz. 0.75 per cent. With this sole exception the values range from 0.5 per cent. downwards, the surface being usually entirely devoid of detectable carbonates.

The average is 0.01 per cent. by weight or 0.1344 grm. per cubic decimetre. Comparison of B 8 and B 9, and B 13 and B 14, brings out clearly the leaching effect in the vertical direction, and emphasizes the almost complete absence of carbonates from the surface layer.

The Hood, though presenting a still older system than the Long Hills, yet shows the same range and average content by weight as the latter (viz. 0.01). This we can attribute to its position, some 160 yds. from the foreshore, which facilitates a small amount of accretion. Despite this, the greater degree of leaching is brought out when we compare the weight of carbonate per unit volume, which here amounts to only 0.1281 grm. per cubic decimetre.

It is clear that for any given plant with its specific capacity for root development, the amount of carbonate in a given volume of soil is the important consideration.

The generally xerophytic character of the dune flora is so well known as to require no emphasis, but these same transpiration checks which enable the dune plants to retain their foliage during the drought conditions of summer¹ also involve a smaller intake of the soil solution during the moist conditions of spring and autumn. To appreciate fully therefore the significance

¹ During the exceptionally dry summer of 1921 the foliage of several dune and shingle species suffered considerably. Notable examples were: *Silene maritima*, *Frankenia laevis*, *Erodium neglectum*, and *Convolvulus soldanella*.

of the differences observed we must take into consideration the water-content. Data respecting the natural water-content of the different dune phases over a sufficient period are not available, but assuming that these would be more or less proportional to the maximum water-contents observed under laboratory conditions, it will be realized from a perusal of Table IV that the differences in concentration must be very pronounced.

TABLE IV.

Summary of the Carbonate Content of the various Dune Types.

<i>Dune type.</i>	<i>Avg. carbonate content by weight.</i>	<i>Avg. carbonate content per cubic decimetre.</i>	<i>Avg. concentration of carbonates at maximum water-content.</i>
	%	gm.	%
'Yellow' dunes { Embryo dunes J and G	0.425	6.077	1.697
{ Main ridge E	0.341	4.8763	1.343
'Grey' dunes { Lab. ridge D	0.155	2.139	0.660
{ Long Hills B	0.010	0.1344	0.034
{ Hood A	0.010	0.1281	0.027

The figures in the third column represent the average concentrations *if all the carbonates present were in solution* at once, so that they have little significance except in their relation to one another.

If we take the embryo dunes as unity then the relative accessibility of carbonates, on the assumption that the solubility in carbonated water is proportional to that in dilute HCl, is approximately as follows: embryo dunes, 1; main ridge, 0.8; Laboratory ridge, 0.33; Long Hills, 0.02; Hood, 0.016. As the amount of carbonate present is the limiting factor for the amount dissolved, the values realized in nature would show an even steeper gradient than these figures indicate.

The organic content of the dune soils (cf. Table I, Appendix, and Table V).

Embryo dunes. The outstanding feature of the early phases, apart from their mobility, is the low proportion of organic material, probably almost entirely derived from drift.

In the fourteen localities for which the loss on ignition of 'embryo' dunes was determined, the range was from 0.16 per cent. to 0.52 per cent., and it is significant that the highest value was obtained from a very young dune near the drift line. The average value is 0.360 per cent., or approximately 5 gm. per cubic decimetre.

The main ridge. Here, associated with the denser vegetation and greater lapse of time, the organic content ranges from 0.24 per cent. to 0.69 per cent., whilst the average value (12 loci) was found to be 0.501 per cent., or approximately 7.1 gm. per cubic decimetre.

The ridges C and D. The vegetation here has become an almost con-

tinuous carpet, and the organic range is between 0.30 per cent. and 0.96 per cent. The average for seventeen localities is 0.525 per cent., or approximately 7.2 gm. per cubic decimetre. The highest values are associated with a continuous covering of *Cladonia* (C 41, C 42) or moss (D 36 a-c, D 39, D 1). If the two estimations for ridge C may be taken as typical, then the organic content here rises to nearly 12 gm. per cubic decimetre.

Long Hills. The lowest value on these still older dunes correspond with the average for ridge D, whilst the average for the Long Hills is almost exactly double, viz. 1.154 per cent. or about 15.5 gm. per cubic decimetre. The observed range was from 0.56 per cent. to 2.69 per cent., the highest value being associated with the occurrence of *Polypodium vulgare*.

The Hood. As already indicated, this is subject to some accretion, and there are, moreover, numerous rabbits which continually disturb the normal edaphic relations by bringing to the surface the less organic sand from below. This factor operates throughout the dune area, but especially on the Long Hills and Hood. Despite these disturbing influences, the surface soil of the Hood shows a much higher organic content, ranging from 0.61 per cent. to as much as 6.34 per cent., with an average of 2.69 per cent., or nearly 34.5 gm. per cubic decimetre.

A thirty-year-old pine wood on Holkham dunes showed an average organic content of 13.2 per cent., or over 150 gm. per cubic decimetre, in the first four inches of soil.

The dunes of successively greater age thus exhibit a perfect gradation in organic content, so that when more dune systems have been examined in detail, it may be possible to determine their approximate age by this means.

If we represent the organic content of the youngest dunes by unity, the proportional values are approximately as follows: embryo dunes 1; main ridge, 1.3; Laboratory ridge, 1.4; ridge C, 2.3; Long Hills, 3.0; Hood, 6.7.

TABLE V. *Summary of Data respecting Organic Content of Dunes.*

Region cf. map)	J and G	E	D	C	B	A (oldest phase)
Av. %	0.360	0.501	0.525	0.86?	1.154	2.69
Weight per cubic decimetre	5.148	7.164	7.245	11.868?	15.509	34.459

The appended data (Table VI), obtained from a transect of three samples from the Southport dune system, indicate that the same type of sequence in edaphic phenomena probably characterizes dune systems in general.

TABLE VI. *Transect Southport Dunes (Samples 0-6 in.).*

	Water capacity.	p. H.	Total carbonates. %	Organic content. %	Silt + clay. %
Dune near sea face	23.5	7.4	3.5-4.85	0.30	0.34
Medium aged turf-covered dune	30.3	7.1	2.1-3.65	2.92	1.07
Old dune near fringe of cultivation	44.0	6.8	0.5-1.6	3.25	1.10

Water-content.

Since the mineral particles are fairly uniform as regards the relative proportions of the mechanical fractions in the different phases of the dune system, the variations in water-content are mainly dependent on the proportion of organic material present.

The subjoined table (Table VII), in which the maximum water-content is shown, brings out quite clearly how this increases with the increasing age of the dune system.

TABLE VII. *Maximum Water-contents.*

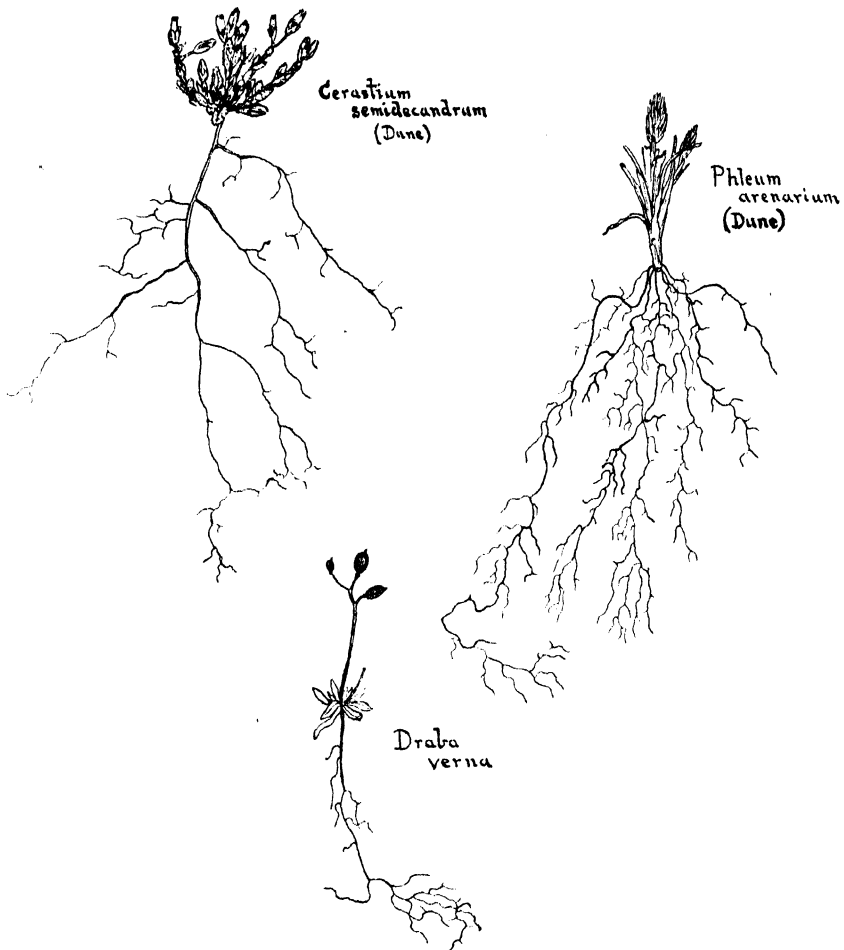
<i>Dune phase.</i>	<i>Water by weight, %</i>	<i>Water by volume, %</i>	
Main ridge	26.6	38.04	} Av. by weight 25.4 % " volume 36.3 %
"	26.4	37.75	
"	24.8	35.49	
"	20.3	29.0	
"	24.0	34.3	
"	25.3	36.1	
"	27.6	39.5	
"	26.0	38.0	
"	27.0	38.6	} Av. by weight 29.46 % " volume 39.44 %
Long Hills	28.2	38.0	
"	28.9	38.8	
"	28.9	38.8	
"	26.7	35.87	
"	29.0	39.88	
"	32.14	43.20	
"	30.1	40.45	
"	28.2	38.00	} Av. by weight 35.90 % " volume 45.99 %
"	31.2	41.00	
The Hood	43.0	55.08	
"	34.3	43.94	
"	34.5	44.19	
"	37.3	47.80	
"	29.8	38.20	
"	32.54	41.70	
"	33.2	42.53	
"	31.9	40.86	
"	46.59	59.68	

Comparing the average water-contents in Table VII with the average organic contents already given in Table V, we find that the Long Hills show an increased water-content over that of the main ridge of 3.06 per cent., whilst the increased water-content of the Hood as compared with the main ridge is 10.50. The respective increases in organic content are 0.653 and 2.19.

If then the increase of the water-content is due almost entirely to the increased organic material, it follows that we should be able to calculate approximately the increase of water content for the Hood from the organic increase, having regard to the figures yielded by the Long Hills. This calculated value is 10.26 instead of 10.5 as found experimentally. Put in another way the ratio $\frac{\text{increase of water-content}}{\text{increase of organic content}}$ should yield an approximate constant; the actual values obtained are 4.68 and 4.79, which are as close as could be expected.

The conclusion seems warranted, then, that the organic material is

mainly responsible for the water capacity of dune soils, and it is in conformity with this that the roots of ephemerals (cf. Text-fig. 2) tend to occupy the upper layers of the soil in which the humus is mainly present. Also it is significant that numerous rootlets are often developed around old buried rabbit droppings with which the root systems may come in contact.

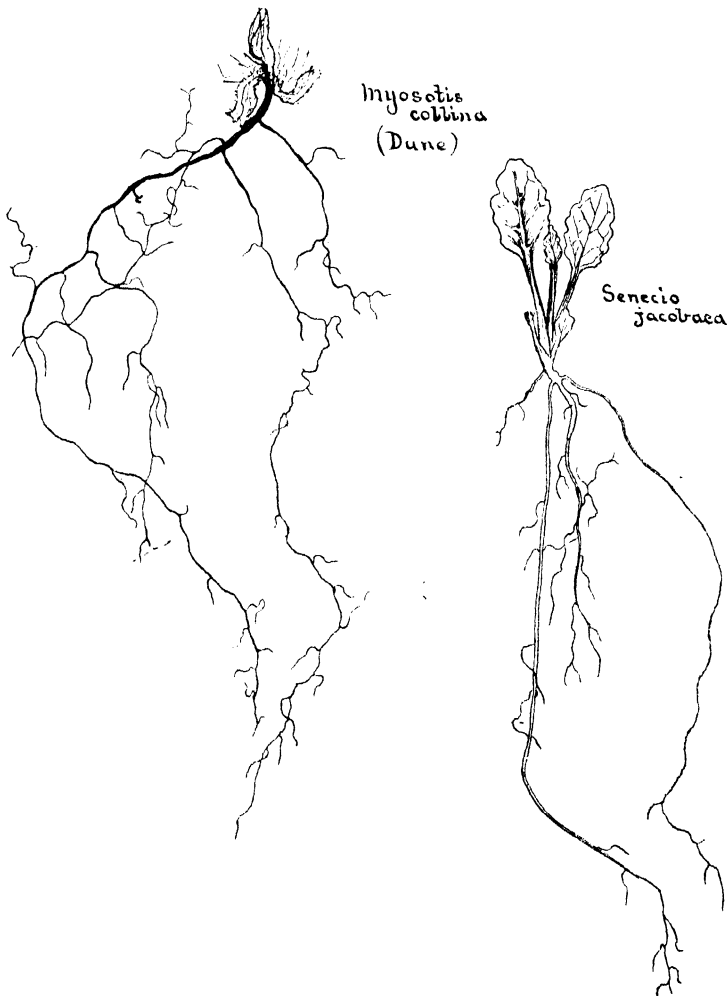


TEXT-FIG. 2. Root systems of *Cerastium semidecandrum*, *Phleum arenarium*, and *Draba verna*, three dune ephemerals, showing their shallow character. ($\frac{2}{3}$ natural size.)

The subject of the nature and extent of root systems cannot be gone into here, but it may be mentioned that careful study of those of several ephemeral species shows that the volume of soil exploited is about 360 c.c. in *Cerastium semidecandrum*, 160–430 c.c. in *Phleum arenarium*, 750 c.c. in *Myosotis collina*. In most cases the average depth of the root systems of the ephemerals is about 12 cm.

In contrast to this, the larger plants have very extensive and deep

root systems on which one not infrequently notes the copious development of fine rootlets in relation to rabbit faeces, fragments of old *Psamma* rhizomes, &c. For example, the root system of a *Silene maritima* plant growing in sand occupied a volume of over eight cubic feet of sand, and the aggregate length of the main roots was over 11 ft. (For the exposure



TEXT-FIG. 3. Root systems of *Myosotis collina* and a seedling of *Senecio jacobaea*. ($\frac{1}{3}$ natural size.) of this root system I am indebted to Miss P. Hutchinson.) Here, as in other cases, the method adopted was to support the roots *in situ* by means of wire stakes and remove the sand by an air blast.

Soil Reaction and the Effect of Rabbits.

The highest p. H. values in the dunes, i. e. the most alkaline conditions, are realized in the earliest stages of the succession. The lowest p. H. value observed in the young dunes was 6.9 and the highest 7.4.

The average for all the thirty different loci sampled was 7.17.

The sand in these early stages is therefore generally alkaline and only rarely slightly acid.

The main ridge shows a similar range, viz. p. H. 6.9–p. H. 7.3, whilst the average for twenty-six localities is slightly more acid than for the youngest phase, being 7.029.

Transferring these values from the logarithmic notation to specific alkalinities, the average value for the young dunes is represented by a specific value of 1.5, and that of the main ridge by approximately 1.

Determinations of the ridges D and C made in samples from twenty-five loci showed an average value of p. H. 6.4 for the 0–4 in. depth, or a specific acidity of approximately 3.98. The values range from p. H. 6.1–p. H. 7.3, whilst for the top inch the p. H. may be as low as 5.6.

Several series were taken at varying depths (cf. C 20–C 35; D 12–D 9; D 13–D 19; D 37–D 38). These represent nine locations, and in eight show a lower value for the surface inch. The rise in acidity from below upwards is somewhat abrupt and corresponds to the region of rapidly increasing organic content (cf. D 17, D 18).

On the Long Hills the range is from p. H. 5.9–p. H. 7.0, with p. H. 6.38 as the average value for the top four inches (fourteen locations), a slightly higher acidity than that of ridge D. Here, too, the surface layer is the most acid. B 19, B 20 offer, it is true, the reverse condition, but these are samples from the landward slope of the proximal end where limited accretion takes place.

The Hood samples show a p. H. range of from p. H. 5.5–p. H. 6.9, with an average of 6.24, corresponding to a specific acidity of about 6.3, or rather more than $1\frac{1}{2}$ times as acid as the ridges C and D.

It is then evident that the acidity of dune soils increases with their age. The presence of carbonates in appreciable amount in the early phases of dune development obviously inhibits at first the realization of an acid reaction. But, as we have already shown, the carbonates diminish owing to continued leaching action as we pass from the younger to the older dunes, so that in the latter the rising acidity develops unhindered.

If we compare only those dune soils of ridges C, B, and A, in which no detectable carbonates are present, we find that the averages still show the same increasing acidity, viz. ridge C, p. H. 6.83; B, p. H. 6.28; Hood, p. H. 6.07. Here, then, the rise is clearly not related to carbonate content, but is associated with increasing organic content accompanying increasing age.

Comparison of the samples from the Long Hills amongst themselves shows that the lowest p. H. value is associated with the highest organic content, but the lowest organic content is not associated with the highest p. H. value. That the relation is nevertheless a real one seems indicated by those

samples where both the surface and subsurface agree in having the same carbonate content or none at all. Thus samples D 17 and D 18 from the Laboratory ridge both showed 0.05 per cent. carbonates, but whilst the upper four inches, with an average organic content of 0.53 per cent., had a p. H. of 6.9, the lower level (4-8 in.), with an organic content of 0.35 per cent., had a p. H. of 7.1. The same feature is brought out by the samples B 6 and B 7, and A 1 and A 2.

In view of such results where the humus has the same source of origin, the suggestion seems warranted that such discrepancies as occur between the organic content and the p. H. value, where carbonates are absent, may be, in part at least, attributable to differences of origin of the organic material; in other words, may be influenced by the nature of the plant covering. Also the rate and state of decay are important factors.

Determinations of humus obtained from different species show considerable differences as indicated below :

<i>Psamma</i> 'leaves'	very slightly decayed	p. H. 5.4-6.0
"	" more decayed	p. H. 5.7-6.4
"	" almost completely disintegrated	p. H. 6.2-6.8
<i>Senecio jacobaea</i>	very slightly decayed	p. H. 5.6
<i>Silene maritima</i>	advanced decay	p. H. 7.3
<i>Carex arenaria</i>	partly decayed	p. H. 6.7
<i>Cladonia</i>	" "	p. H. 5.4

These estimations were based on too few examples to permit us to lay much stress on the actual values obtained, but the *Cladonia* 'humus' is clearly very acid, whilst the 'humus' formed by the pioneer species is clearly less so. Within certain limits we should expect the humus derived from a given species to have a definite reaction dependent on the degree of ionization of the substances capable of yielding hydrogen ions. Therefore on first consideration it might seem unlikely that the amount of organic matter present would influence the reaction. But the above data for *Psamma* show that the reaction varies with the stage of decay, and will therefore be influenced by the rapidity with which this proceeds. The amount of humus present is roughly a measure of the rate of decay, and hence a large organic content being associated with slow decomposition will involve a larger proportion of material in the earlier and more acid stage of decay.

Another factor that must be considered is the presence of rabbits, which are constantly feeding on such plants as *Psamma arenaria* and *Carex arenaria*, and whose faeces consist very largely of partially digested fragments of their leaves. The faeces are deposited on the dune surface of all phases, but especially the younger. Here they become buried by subsequent accretion, whilst in the older phases they are swept by the wind into the rabbit holes.

TABLE VIII.
Weight and Number of Rabbit Faeces on Dunes.

I. *Number on surface.*

<i>Dune type.</i>	<i>Area examined (15 counts).</i>	<i>Total no./sq. metre.</i>	<i>Air dried. Total weight/sq. metre.</i>
	sq. m.		gram.
Main ridge slope	0.2655	1039	86.65
" "	"	773	73.60
" top	"	1042	96.70
Average for 'yellow dune' by number, 951 per sq. metre; by weight, 85.6 gm.			
Laboratory ridge	0.2655	926	59.20
" "	"	1090	92.80
Ridge P, Loose sand (<i>Soldanella</i>)	"	952	70.71
" P, <i>Cladonia</i> , moss, <i>Psamma</i>	50	332.6	19.25

Average for 'grey dune' by number, 825 per sq. metre; by weight, 60.40 gm.

II. *Number and weight per unit volume to depth of 7 cm.**Area A.*

<i>Condition.</i>	<i>Volume.</i>	<i>Number per sq. metre.</i>	<i>Air dried. Weight per sq. metre.</i>
	c.c.		gram.
Unenclosed	2,184 { Surface	1,600 } Total 5,470	303.0
	Subsurface	3,870 }	
"	2,184 { Surface	1,248 } " 8,032	60.8
	Subsurface	6,784 }	233.6
"	2,184 { Surface	352 } " 3,072	9.6
Enclosed	2,184 { Subsurface	928 } " 1,280	17.6
"	2,184 { Surface	352 } " 3,360	8.3
	Subsurface	3,008 }	121.3
"	2,184 { Surface	480 } " 3,200	27.2
	Subsurface	2,720 }	118.4

Average unenclosed, 248.2 gm.

" enclosed, 100.8 gm. Difference, 147.4 gm.

Area B.

<i>Condition.</i>	<i>Volume.</i>	<i>Number per sq. metre.</i>	<i>Air dried. Weight per sq. metre.</i>
	c.c.		gram.
Unenclosed	2,184 { Surface	832 } Total 2,656	51.2
	Subsurface	1,824 }	83.2
"	2,184 { Surface	512 } " 5,152	27.2
	Subsurface	4,640 }	213.4
"	2,184 { Surface	1,536 } " 3,104	92.8
	Subsurface	1,568 }	51.2
Enclosed	2,184 { Surface	288 } " 1,888	9.6
	Subsurface	1,600 }	48.0
"	2,184 { Surface	320 } " 1,184	10.2
	Subsurface	864 }	20.8
"	2,184 { Surface	288 } " 3,296	9.6
	Subsurface	3,008 }	112.0

Average unenclosed, 173.0 gm.

" enclosed, 69.8 gm. Difference, 103.2 gm.

" deposit per annum Areas A and B, 35.8 gm. (air dried).

" " (dried at 100° C.) = 34 gm.

" " organic material/100 gm. soil = 0.18.

" content faeces per 100 gm. soil (unenclosed) subsurface A and B = 4.6 %.

N.B.—In the above figures only those faecal particles retained by a 0.5 mm. sieve are included. In the soil data for humus content these have almost entirely been removed by sieving.

The amount of organic material contained in these rabbit droppings is important, as also their reaction. With respect to the former point numerous counts were made of the number of rabbit droppings on a surface of unit area, and also of those present on and below the surface of a unit volume (cf. Table VIII). The figures for the surface deposit were also checked by entirely denuding an area of 50 sq. metres of all the rabbit faeces visible to the unaided eye (cf. below).

It will be realized from the data in Table VIII that rabbits play no small part in the supply of organic material to the soil.

Comparing the figures for the open dune, and those for areas of the same dune from which rabbits had been excluded for a period of $3\frac{1}{2}$ years, we arrive at the conclusion that the annual deposit (assuming the rate of decay of the old droppings to be of the same order as for old and new together in the unenclosed area) is approximately 34 grm. on an area of 312 sq. cm., or about 0.18 per cent. by weight of the soil.

A striking feature of the decay of these faeces is that their form is more or less retained throughout the process, so that one can roughly grade them according to age by the change in size, as well as by the progressively darker tint.

Estimations of the hydrogen-ion concentration show that as decay proceeds there is a similar change with age as observed for normal plant remains, the early stages being much more acid, p. H. 5.8-p. H. 6.4 (av. p. H. 6.1), than the most advanced state of decay (av. p. H. 6.9). Despite this difference, however, and the comparatively low buffer action of sand dune soils, there is only a slight difference in the reaction of the enclosed and unenclosed areas. Such difference as exists is indeed the reverse of what one might expect from the absence of recent droppings in the enclosed areas (cf. Table IX).

The clear tendency, as shown in Table VIII, 1, is for the rabbit droppings to decrease in amount from the younger to the older dunes (compare 'yellow' and 'grey' dunes, Table VIII) in correspondence with the diminishing amount of *Psamma arenaria* and other of the more favoured food plants.

TABLE IX.

Area A.

Enclosed (Sample I)	p. H. 6.85	Unenclosed (Sample I)	p. H. 6.95
" (" II)	" 6.85	" (" II)	" 6.95
" (" III)	" 6.80	" (" III)	" 7.25

Area B.

Enclosed (Sample I)	p. H. 6.9	Unenclosed (Sample I)	p. H. 7.0
" (" II)	" 6.9	" (" II)	" 6.9
" (" III)	" 6.9	" (" III)	" 6.9

Av. enclosed p. H., 6.86; av. unenclosed p. H., 6.95

As the acidity and organic content of the soil both rise with increasing age of the dune system, the influence of rabbit droppings, either as regards the amount of organic material added, or the soil reaction, is evidently of quite minor importance. Rabbit faeces are, however, of considerable significance in the water economy of the dune plants, as is shown by the frequency with which copious development of fine roots is often associated with their presence.

The Influence of the varying Soil Conditions on the Dune Vegetation.

The stages in dune succession have been shown to present a graduated series of conditions with increasing age; an edaphic succession in fact in which mobility, low organic and water contents, high calcium content, and a neutral or alkaline reaction mark the earliest phases, whilst the final phases present a stable soil, a high organic content, relatively high water-content, a total absence or negligible quantity of calcium, and an appreciable acidity. The adaptation of the pioneer species to mobile soil, the stimulated growth they exhibit when buried by further accretion, are facts so well known as to require no emphasis here. The stabilizing action of the plant covering, which enables less specialized species to become established, is also not only familiar but underlies the elaborate technique of dune maintenance and protection. The calcium content and reaction of dunes have, however, received but little attention, and it is upon the influence of the changes in these that we would lay particular stress.

The writer has elsewhere pointed out ('The Significance of the Calcicolous Habit', *Journal of Ecology*, vol. viii, pp. 202-15, 1920) that the plants normally characteristic of soils rich in calcium probably occur on such, either on account of their preference for bases or because of the dry character of these soils. The data here given show that both conditions are satisfied in the younger dune phases, and hence we might reasonably expect to find something in common between the flora of the chalk and that of the 'yellow' dune.

There is reason for suspecting that some of the pioneer species, such as *Psamma arenaria*, *Elymus arenarius*, *Agropyrum junceum*, *Euphorbia Paralias*, &c., are somewhat partial to a calcium-rich medium and may perhaps be 'oxyphobic', but their specialization to a mobile substratum precludes the expectation of their occurrence on chalk downs and, similarly, we should not expect to find representatives of the calcareous pasture on the extremely mobile soil of an embryo dune. The edaphic conditions of the later phase of the yellow dune are those where we should look for the resemblances indicated.

Actually no species especially characteristic of the chalk flora are met with on the Blakeney Point dune system, but elsewhere in this country and in other parts of Europe the calcicole element in the dune flora is

a marked feature. Robert Smith describing the fixed dunes near Edinburgh mentions the occurrence of *Thalictrum minus*, *Anthyllis Vulneraria*, *Astragalus danicus*, *A. Glycyphyllos*, and *Gentiana Amarella* (Scottish Geog. Mag., vol. xvi, pp. 385-416, 1900). The list given by W. G. Smith for the dunes on the coast of Fife includes *Astragalus danicus*, *Ononis repens*, *Linum catharticum*, *Trifolium procumbens*, *Medicago lupulina*, *Galium verum*, *Gentiana campestris*, *G. Amarella*, *Thymus serpyllum* and *Koeleria cristata* (Scottish Geog. Mag., vol. xxi, pp. 70-1, 1905). Moss, describing the dunes of Somerset (Journ. Roy. Geog. Soc., pp. 8-17, 1907), enumerates several dune species which attain their greatest abundance on chalky soils, of which *Iris foetidissima*, *Carlina vulgaris*, *Anthyllis Vulneraria*, and *Inula Conyza* are perhaps the most noteworthy. In 'Types of British Vegetation' *Anthyllis Vulneraria*, *Chlora perfoliata*, *Gentiana campestris*, *G. baltica*, *Epipactis latifolia*, and *Orchis pyramidalis* are mentioned as occurring on the Lancashire dunes.

In Ireland the same feature holds. Colgan and Scully in the 'Cybele Hibernica' (2nd ed., 1898) cite many species as calcicolous in that country, of which nine are mentioned as occurring on sand dunes. These are *Arabis hirsuta*, *Viola hirsuta*, *Orchis pyramidalis*, *Ophrys apifera*, *Asperula cynanchica*, *Carlina vulgaris*, *Leontodon hirtus*, *Gentiana verna*, and *Clematis Vitalba*.

As might be expected this feature extends also to the Cryptogamic flora, and Watson (Journal of Ecology, pp. 126-42) cites *Barbula tophacea*, *Trichostomum crispulum*, *Camptothecium lutescens*, *Pellia fabbroniana*, *Preiszia quadrata*, *Lophozia badensis*, and *Scapania aspera*, as species whose occurrence on dunes is determined by the presence of comminuted shells.

On the Continent the same feature is strikingly exhibited. Massart ('Essai de Géographie botanique,' pp. 390-1, 1907) cites no less than twenty species which in Belgium are practically confined to dunes and calcareous soils. Of the total of 117 flowering plants cited by Abromeit ('Handbuch des deutschen Dünenbaues', Berlin, 1900) as present on German dunes some 19 specimens are more or less marked calcicoles. The same feature in another field is exemplified by the occurrence of the calcicole snail, *Cyclostoma elegans*, on sand dunes (cf. A. E. Boycott, Proc. Malac. Soc., vol. xiv, p. 128, 1921).

At the other extreme the oldest dunes present, as we have seen, an almost entire absence of carbonates, and it is associated with this condition and a high acidity that *Polypodium vulgare* is met with in our area on the Long Hills, whilst *Pteris* and *Athyrium* occur on the Hood.

On other systems these oldest phases of leached dune soils are marked by the presence of *Calluna vulgaris* and other ericaceous plants. Any factor tending to accelerate leaching will naturally favour the colonization of 'calcifuge' plants. As the writer has pointed out, in relation, particu-

larly, to other types of vegetation (cf. *Journal of Ecology*, vol. ix, pp. 220-40, 1922), leaching being most rapid at the crest of a hill or the upper part of a valley slope, the flora tends as a rule to become more calcifuge in character as we ascend. It is in conformity with this generalization that, as pointed out by W. G. Smith for the dunes of Fife, *Calluna vulgaris* and *Erica cinerea* occur more particularly towards the crest of the dune ridges (loc. cit., p. 71).

Even in these old dunes, with their low proportion of plant food, the hollows support a type of vegetation which bears much the same relation to the calcifuge flora of the ridges as the path and valley-bottom vegetation bears to that of the general woodland vegetation in an acid oak wood (cf. Salisbury, *Journal of Ecology*, vols. iv and vi, 'The *Quercus-Carpinus* Woods of Hertfordshire').

The extreme calcifuge character of ancient dunes is sufficiently emphasized by the fact that such species as *Corallorrhiza innata*, *Vaccinium myrtillus*, *V. Vitis-idaea*, and *Pyrola rotundifolia* have been recorded from old dune systems in this country. That other dune systems show the same feature as those of Blakeney is illustrated by the data given on p. 400 for a series of samples from the Southport dunes.

The dune soils exhibit the usual humus gradient (cf. Salisbury, *Journal of Ecology*, vol. ix, p. 221), which is well illustrated by the appended data from two areas on the Long Hills, one covered by vegetation and the other bare, as well as by the estimations already furnished.

<i>Depth.</i>	<i>No vegetation.</i>	<i>Vegetation.</i>
in.		
0-6	4.78	3.96
6-12	1.38	1.14
12-18	0.68	0.93

It is this increase in humus in the surface layer that is probably mainly responsible for the colonization of the younger 'fixed' dunes by *Tortula ruraliformis* and other mosses. Although relatively dry in summer the surface soil, from its comparatively high organic content (cf. D 36-D 48, D 43, &c.), ensures considerable moisture during the winter months.

In the Blakeney system *Triticum junceum*, *Arenaria peploides*, *Festuca arenaria*, and *Eryngium maritimum* are more or less restricted to the earlier 'yellow' dune phases. In the more stabilized condition *Psamma* decreases in amount and the individuals exhibit diminished vigour.

On the ridge D mosses, particularly *T. ruraliformis*, *Carex arenaria*, *Erodium*, &c., make their appearance.

THE LATERAL SHINGLE BEACHES.

From the point of view of edaphic succession it must be borne in mind that the main shingle bank throughout its entire extent is more or less subject to (a) addition of new material, (b) removal of material from the sea-face landwards, (c) submergence by excessively high tides bringing with them new supplies of organic material and carbonates in the form of shells. These conditions maintain the main beach in a more or less juvenile state, so that the natural changes which might be anticipated with the lapse of time tend to be masked.

The instability of the shingle is considerably diminished by the presence of *Suaeda* bushes, and where these are most numerous, viz. opposite the marrams, the largest number of species and the most extensive carpet of vegetation anywhere upon the main shingle bank are met with. The importance of this lack of mobility is further emphasized by the location of certain species, e. g. *Convolvulus soldanella* in the neighbourhood of the *Suaeda* bushes, with every indication of having spread from these latter as their centres of origin (cf. F. W. Oliver, 'The Shingle Beach as a Plant Habitat', New. Phyt., vol. ii, pp. 73-99, 1912, and Oliver and Salisbury, 'Vegetation and Mobile Ground', Journal of Ecology, vol. i, pp. 249-72, 1913).

The lateral hooks still further emphasize the great importance of the degree of mobility. These are for the most part extremely stable and present in their older phases a continuity of vegetation which is never attained on the main bank itself.

Each lateral bank represents a landward deflexion of an original termination of the main bank, which latter subsequently continues its growth in a sympodial manner.

The interval between two successive laterals is thus an indication of the time interval between the violent storms during which the laterals are formed. The latter, owing to the continued growth of the main beach, are relatively sheltered, and this protection is increased with the formation of each new lateral (cf. Oliver, 'The Shingle Beach as a Plant Habitat').

Mechanical Analysis and Mobility.

Perusal of the data in Table X respecting the mechanical analysis of the successive shingle laterals into coarse and fine particles shows that the proportion below 0.5 mm. in diameter increases with the increasing age of the shingle bank, the lowest values being obtained in samples from the main beach. The oldest laterals have indeed nearly seven times the proportion of fine particles present in the shingle of the main beach.

It will be noted that both the observed range and average for the oldest lateral but two (Bank III) is higher than for the oldest lateral itself.

This, taken in conjunction with the higher carbonate content and lower acidity of the latter, is indicative of an edaphic condition not strictly in conformity with its position in the series.

The increasing proportion of fine material on the older banks naturally tends to cement together the larger stones more firmly, thus effectively contributing to the augmenting stability. In addition, it further reacts on the power of water retention, so that plants less tolerant of both drought conditions and mobility are able to colonize the shingle. (For a discussion of the water relations of shingle beaches, cf. Hill and Hanley, *Journal of Ecology*, vol. ii, pp. 21-35, 1914.)

TABLE X.

Mechanical Analysis of Shingle.

Percentage of fine particles below 0.5 mm. in diameter.

<i>Main shingle bank.</i>	<i>Range observed.</i>	<i>Average.</i>
	%	%
(a) Bare shingle	2.7-10.6	6.7
(b) Under <i>Suaeda</i> bush	2.8-8.6	5
(c) Fine shingle with grasses	0.4	—
(d) Fine shingle bare	0.19	—
<i>Lateral banks.</i>		
Bank XVII	18-19	18.7
„ XII ('Yankee')	11-30	19
„ VIII ('Watchhouse')	28-31	29
„ VII	27-32	30
„ IV	20-35	26
„ III	35-60	47
„ I (oldest)	33-56	45

The two chief pioneers, *Arenaria peploides* and *Silene maritima*, are both 'mat' formers which react freely to burial under shingle and are stimulated to more vigorous growth thereby. Both freely develop adventitious roots, and *Silene maritima* not infrequently adventitious buds, from the deep-seated and extensive root system.

Any attempt to dig up either of these species will convince the most sceptical of the extent of root and shoot systems and their efficacy as shingle binders.

When NW. gales combine with high tides the main shingle bank may at very infrequent and irregular intervals become awash, and in these conditions the soil fractions become to some extent elutriated. The finest particles are probably carried down to the landward side or on to the marshes, the heaviest pebbles may be moved only slightly, whilst the fine shingle bestrews the floor of the percolation gully. Therefore such shingle patches, as will be seen from Table X (c) and (d), contain an extremely low proportion of particles under 0.5 mm., and only about 27 to 31 per cent. over 6.5 mm., as compared with 35-61 per cent. elsewhere. This water-sifted shingle is the especial home of *Poa loliacea*, *Lepturus filiformis*, and *Sedum acre*, whilst the most stable shingle of the main beach supports the

densest growth of *Silene maritima* and *A. peploides*, with the addition in particular of *Sonchus arvensis* v. *angustifolius*, *Festuca rubra*, three isolated patches of *Convolvulus soldanella* and two of *Tussilago Farfara*. The latter is often regarded as characteristic of stiff clayey soils, but its absence from many lighter types is very probably an outcome of their poverty in mineral salts and acid tendency. Unless it be the conditions associated with a more or less neutral reaction that determine the occurrence of *T. Farfara* its presence here demands explanation.

Organic Content, Carbonates, and p. H.

In order to appreciate the significance of the data regarding the organic content, carbonate content, and hydrogen-ion concentration, it is necessary to appreciate that, just as the main dune ridge, which marks the oldest range with marked accretion, is the highest, so too the shingle laterals develop to a maximum, after which they undergo a flattening process partly perhaps the outcome of wave action, but largely the natural process of 'settling' which manifests itself chiefly in the replacement of the convex crest by a flat one. The highest level reached by laterals is at their L-shaped distal end, which forms a high elbow here, although only reached by the highest tides during exceptional storms; the same flattening of the profile is to be noted.

As a consequence of this lowering of the level with age, the oldest lateral banks share in common with the younger the fact that they are more frequently tide-covered than those of intermediate age; it is to this that we must attribute the attainment of the most extreme conditions associated with increasing age on the Watchhouse bank and high elbows.

The Carbonate Content.

The data in Table XI, Appendix, and the appended summary Table XII, show that the total carbonates are highest in the youngest lateral XX and fall to their minimum in laterals IV to VIII, again rising slightly in I and III.

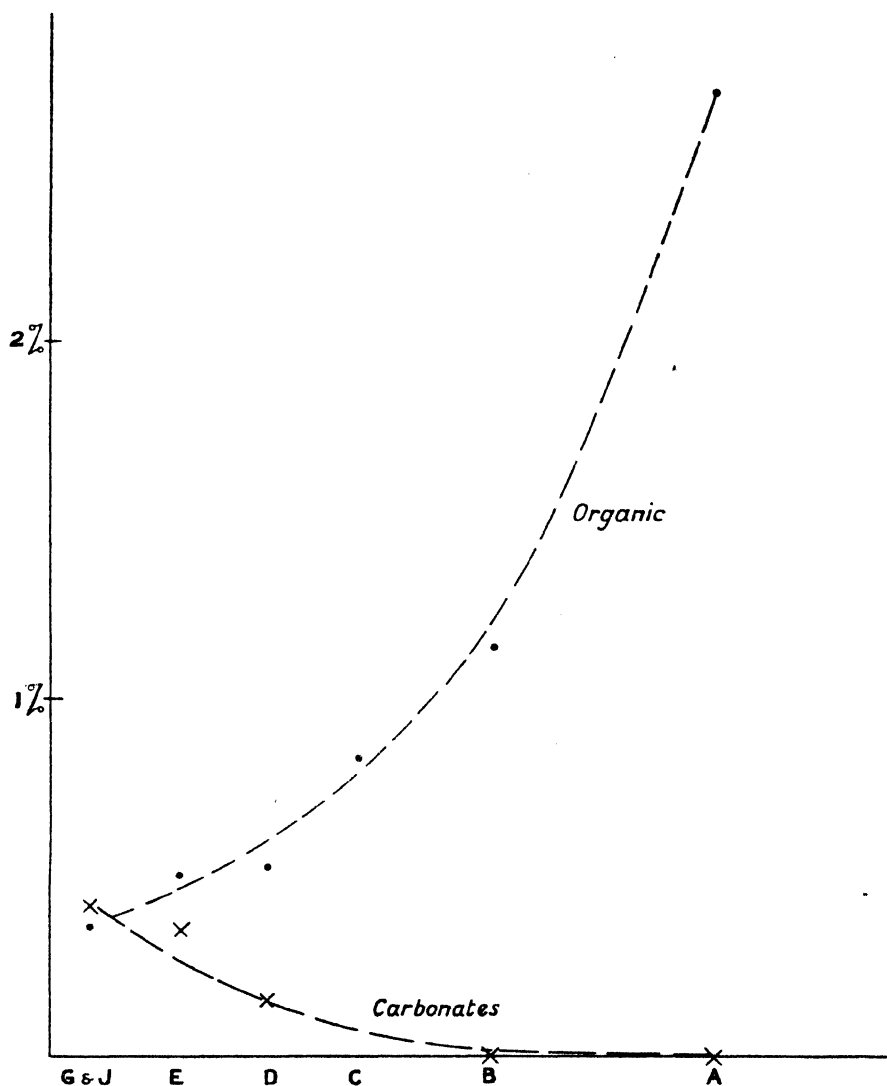
TABLE XII.

Summary of Data for Crest of Laterals.

	(Averages of all estimations.)								
	Youngest bank = XX; oldest = I.								
	XX.	XVII.	XVI.	XII.	VIII.	VII.	IV.	III.	I.
Av. loss on ignition %	0.486	0.32	—	7.12	22.45	5.52	2.39	2.64	3.19
„ total carbonates %	0.813	0.16	—	0.103	0.03	0.03	0.00	0.006	0.015
„ p. H.	7.59	7.22	7.0	6.72	6.38	6.59	6.96	6.86	6.90

Here then, too, as on the older dunes, we see the effect of leaching clearly marked. In the case of Bank VIII the two spots on the high elbow

(X.46 and X.46a) show a lower carbonate content than the main bank, but the single sample from the high elbow of VII shows a slightly higher



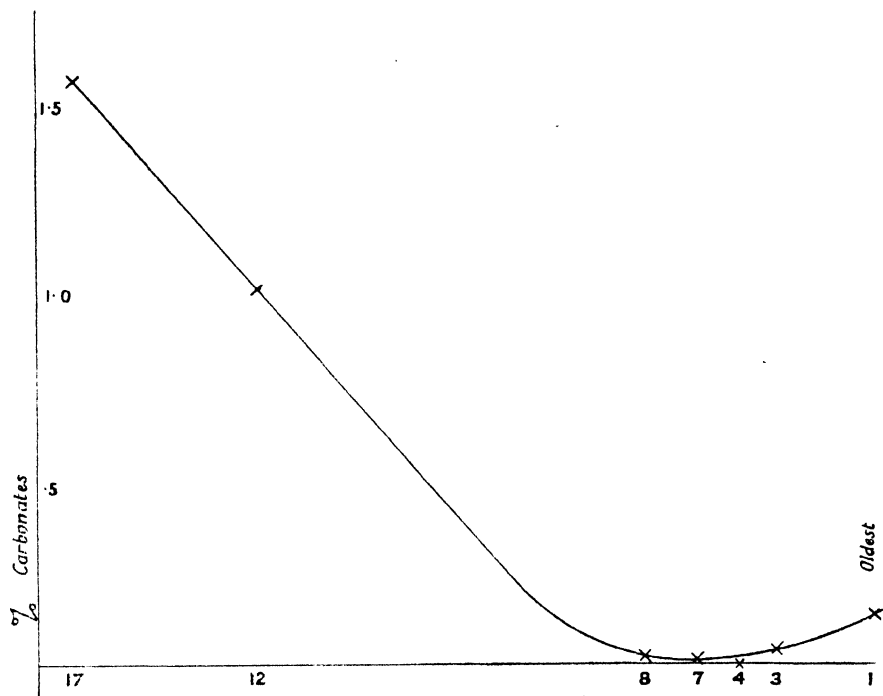
TEXT-FIG. 4. Graph showing the relation between organic content and carbonates in dunes of varying ages as indicated by the letters A to J (cf. map, Text-fig. 1).

content than the other parts of the bank. Taking all the banks into consideration, however, there is a general tendency for the lowest values to be found towards the higher levels at the distal end, which supports the view that leaching is the main factor involved.

The Organic Content.

The youngest bank has a very low organic content, under a half per cent., whilst again the eighth lateral shows the extreme condition with an average of over 22 per cent., and there is falling off in the older laterals.

Here, then, it is quite evident that the increasing organic content accompanying increasing age is a feature of the lateral shingle banks as well as of the dunes. That the diminished organic content of the oldest



TEXT-FIG. 5. Graph showing relation between percentage carbonate content of the soil of the crest of shingle laterals and their age as indicated by their positions in the shingle system.

laterals is correlated with their lower level is borne out by the exceptionally high organic content shown by the soils of the 'high elbows' of both laterals VIII and VII. In the former the organic content may be nearly 29 per cent., and in the latter nearly 15 per cent. as compared with about 12 per cent. and 4 per cent. as the respective observed maxima for the lower parts of the crest.

The Hydrogen-ion Concentration (cf. Tables XI and XIII, Appendix).

As is to be expected from the foregoing considerations, the reaction of the soil of these laterals exhibits an increasing acidity with age, which attains its maximum on the high elbow of Bank VIII with a p. H. of 5.9. The

averages show a perfect gradation from XX to VIII, with a subsequent decrease in acidity (rise of p. H.) in the oldest laterals.

It is evident that the inundation of these latter, infrequent though it be, is sufficient to restrain the development of a high acidity, and hence by favouring the oxidation of the organic material precludes the accumulation of a high proportion of humus.

The crest of the lateral banks, therefore, presents us with a definite edaphic sequence of the same general type as that shown by sand dunes or by woodlands, but whereas in these cases the increasing acidity would appear to be more or less continuous till the curve flattens out, here a topographical change intervenes to cause retrogression.

Vegetation (cf. Appendix, Table XIV).

The high elbows of VII and VIII exhibiting as they do the maximum of *both* height and age, we not unnaturally find here the greatest departure from the maritime condition. The less specialized habitat, consequent upon the increased proportions of organic material and finer soil particles, and the still greater freedom from tidal inundation, associated as these are with increased stability and the operation of the time factor, brings with it an augmented flora. It is interesting in this connexion to note that, as shown in the accompanying lists (Appendix, Table XIV) for laterals of varying age, the maximum number of species, viz. fifty-one, is recorded for Bank VIII, whilst the smallest number, viz. six, is present on the newly-formed lateral. That the time factor alone is not responsible is shown, however, by the diminution in number on the oldest laterals as shown below.

Number of Species present on Crest of Laterals of Increasing Age.

	Youngest ← ————— → Oldest								
	XX.	XVII.	XII.	VIII.	VII.	VI.	IV.	III.	I.
No. of species	6	6	34	51	36	25	21	24	16

On the youngest phases several species are present whose occurrence may be attributed to their proximity to dunes. This especially applies to Bank XII, where alone we find *Phleum arenarium* and *Stellaria boreana* occupying a shingle crest. Other species which are characteristic of the earlier phases and absent from the oldest are *Senecio jacobaea*, *Rumex crispus* v. *trigramulatus*, *Glaucium luteum*, and *Arenaria peploides*.

Silene maritima, although common on the oldest banks, is yet not so abundant as on those of medium age, nor are the individuals so vigorous.

Amongst the species confined to the 'high elbow' are *Galium verum*, *Trifolium procumbens*, and *Vicia angustifolia*. These, together with *Aira praecox*, *Festuca ovina*, *Plantago Coronopus*, *Trifolium arvense*, *T. striatum*,

Rumex Acetosella, and *Senecio sylvaticus*, and several others, are frequent and characteristic species of some of the gravelly heaths inland. *Rumex Acetosella* in particular, and the abundant *Cladonia* on all the older banks, serve to illustrate the tendency on the older banks to develop an acid-tolerant type of vegetation.

The Flanks.

Occupying the slopes of the laterals on either side there are several zones, of which the most conspicuous is the *Suaeda fruticosa* zone clothing the lower edge, and more or less corresponding in position to the normal zone of drift deposition. Above the *Suaeda* zone is a zone of rather open vegetation with numerous bare pebbles, and characterized by the presence of *Statice binervosa* and *Frankenia laevis*. Locally, at the upper limit of this zone where it passes into the crest, sometimes at the upper limit of the *Suaeda* zone, but probably always where drift tends to be deposited by the highest tides and during storms, we find a zone in which *Triticum pungens* is a marked feature, sometimes accompanied by *Atriplex littoralis*.

Within the upper portion of the *Suaeda fruticosa* zone, particularly on the basiscopic side of the banks where there is more shelter and the slope is gentler, *Festuca rubra* may form an interrupted zone, whilst on this side also *Artemisia maritima* frequently occupies the lower margin of the *Suaeda* zone.

As might be expected, both the *Suaeda* and the *Triticum* zones show a high organic content, three localities in the former yielding a range from 4.9 per cent. to 15.7 per cent. (cf. also marsh vegetation, p. 418), whilst a similar number for the *Triticum* zone showed a range of from 6.38 per cent. to 41.5 per cent. The corresponding hydrogen-ion estimations ranged from 7.2-7.4 for the *Suaeda* zone and 7.0-7.6 for the *Triticum* zone. From the marrams area only one carbonate determination was made for the *Suaeda* zone, viz. 2.55 per cent., though elsewhere on the area *Suaeda fruticosa* occurs in soil with as low a value as 0.26 per cent. In the *Triticum* zone the total carbonates range from 0.04 to 4.2 per cent.

On the whole, then, the *Suaeda* and *Triticum* zones, that is, the zones of drift deposit, show a high organic content and an appreciable or often considerable proportion of carbonates.

In contrast to these, the *Binervosa* zone has a low carbonate content, varying from 0.01 to 0.07 per cent. (av. 0.028 per cent.), whilst the highest organic content observed was 4.91 per cent. and the lowest 0.47 per cent. (av. 2.63 per cent.). Appendix, Table XVI, shows also that the chloride content is subject to considerable variation in one and the same bank. These facts, combined with the slope of the surface, tend to render the habitat a decidedly xerophytic one, and it is significant that not only are the dominant species pronounced xerophytes, but that *S. binervosa* here

assumes a very dwarf habit in comparison with that of plants growing on the crest, whilst *Plantago Coronopus*, which is found in the normal state on the crest, here assumes the *forma pygmaea* (that this is only a habitat form and not a variety has been experimentally demonstrated by the writer).

The Main Beach.

The data regarding the main beach (Appendix, Table XIII) serve to show its close resemblance as a whole to the younger phases of the laterals. There is, however, a marked difference between the bare shingle and that covered with vegetation. Whereas the average carbonate content of the former is 0.53, that of the latter is 1.39 per cent., whilst the average organic content under vegetation is 2.53 per cent. as compared with 0.99 as the average for bare shingle. Since drift is often carried high up on to the main beach, this naturally tends to collect around the *Suaeda* bushes and on the mats of vegetation, so that the organic material supplied by the plants themselves is augmented by supplies of drift, which also brings with it carbonates in the form of shell fragments. Thus the influence of the plants themselves on the stability and character of the shingle soil tends to be cumulative in its action.

Chloride Determinations.

A few determinations of chlorides for various parts of the area given in Table XVI (Appendix), though insufficient to warrant any detailed consideration, are sufficient to show that the percentage of chlorides is low both for the dunes and the crests of the older shingle banks. The main shingle bank exhibits considerable variation, and indeed these data seem to indicate that the percentage of chlorides, except perhaps in the salt marsh itself, is chiefly significant in relation to the vegetation, as an index of the incidence of tidal inundation.

THE MARSH VEGETATION.

Consideration of the data in Table XV (Appendix) would seem to indicate that the differences in p. H. value have little, if any, significance in relation to the zones of marsh vegetation.

These latter are three in number: the *Suaeda fruticosa* zone, occupying the upper edge flanking the shingle; the *Salicornietum*, occupying the floor of the marsh; and the *Obione* zone, situated on the slight slope between.

Both the averages for all the data and for the transects alone show the same relation to hold with respect to the carbonates and organic matter present, namely maximum values for the *Salicornietum* (organic 9.19 per cent., carb. 2.55 per cent.) and minimum values for the *Suaeda fruticosa* zone (organic 1.36 per cent., carb. 0.676 per cent.), those for the *Obione* zone

being more or less intermediate (organic 2.74 per cent., carb. 0.92 per cent.). The chloride estimations show that the percentage of these may become very high (6.34 per cent.) in the *Salicornietum*, doubtless, as also in the case of the 3.5 per cent. recorded for the large how on the Long Hills, an outcome of evaporation.

The estimations for the *Suaeda* zone on the lateral beaches show that it flourishes in soil with as high an organic content and with a carbonate content as high as the average for the *Salicornietum*. Similarly, the appended values for an old *Obione* marsh on the marrams area obtained by Mr. Hanley indicate that the proportion of organic material is not the determining factor for this species.

	in.		%		%
Old <i>Obione</i> marsh marram	0-4	Organic matter	12.64	Chlorides	0.684
" " "	4-10	" "	7.59	"	0.576
" " "	10-14	" "	3.20	"	0.342

The observed gradient, then, with respect to carbonates and organic material, when we pass from the *Salicornietum* to the *Suaeda* zone on the *Pelvetia* marsh would appear to be important as an indication of the varying conditions rather than of significance in itself.

The differences observed are indications of the change in level and the consequent change in frequency and degree of their tidal inundation. That neither *Obione portulacoides* nor *Suaeda fruticosa* is tolerant of very high salinities is shown by their normal method of occurrence and growth behaviour. The former on old saltings is especially characteristic of the high banks of the creeks and hummocks, whilst the latter not only attains its maximum luxuriance in the upper part of the *Suaeda* zone, but, by the high development of anthocyanin pigmentation which it frequently exhibits in the low-lying areas of its distribution, betrays the existence of conditions which, whilst favourable to the formation of assimilates, are unfavourable to growth—conditions, that is, of physiological drought.

As noted by Professor Oliver at Erquy in Brittany, halophytes are very susceptible to the influence of rainfall (New Phyt., vol. v, p. 190, 1906), so much so, that the extent of growth made would appear to be largely conditioned by whether the precipitation for any given season falls mainly in the periods of the 'neap' tides or the 'springs'. This would seem to show that growth remains more or less in abeyance except during the periods following rain when the soil solution is relatively dilute. The appended data obtained at Erquy not only illustrate the rapid effect of rain in leaching out the chlorides, but also how much this is accentuated by the slightly higher level in the region occupied by the *Glyceria* sward, and still more by the higher level of the raised bank occupied by *Obione*. (During the period of observation none of these zones were reached by the

tide.) Presumably, therefore, the effect is even greater in the *Suaeda* zone. In addition to the enhanced leaching effect which the higher level brings about, the less frequent and shorter duration of inundation increases the probability of the rainfall being efficacious. The views here expressed fully accord with those of R. H. Yapp and D. and O. T. Jones, whose study of the Dovey marshes led them to state that 'the vertical distribution of salt marsh plants . . . depends largely on the frequency and duration of the periods of submergence and emergence respectively' (Journ. Ecology, vol. v, p. 100, 1917).

	Chlorides.		Decrease.	
	Before rain	%	After rain	%
<i>Salicornietum</i>		5.7		
<i>Glyceria-Salicornia-Suaeda maritima</i>	"	3.79-3.27	"	4.19-4.36
<i>Obione portulacoides</i>	"	2.96-3.14	"	2.50-2.08
				0.37
				25
				35
				87

Both the species in question occur high up on the shingle laterals where inundation is a rare phenomenon. As compared with plants similarly situated on the main beach, however, they are low in stature, and not infrequently of unhealthy appearance. Probably the low proportion of mineral salts and the acid reaction are here the limiting factors.

Miss Halket found (Ann. Bot., vol. cxiii, 1915, pp. 143-54) that *Glyceria maritima* grew best when the water contained no Tidman's sea-salt, *S. ramosissima* and *Suaeda maritima* grew best in a 1 per cent. solution, whilst *S. Oliveri* grown in varying concentrations of NaCl showed maximum growth in 2 per cent. solution. Since, in the last instance, sodium chloride was employed instead of the balanced solution furnished by sea-salt, it seems probable that maximum growth would have been exhibited in an even higher concentration of the latter. In any case it is significant that *Salicornia Oliveri* occupied the lowest zone at the Bouche d'Erquy, *S. ramosissima* an intermediate zone, and *Glyceria* the highest parts.

The *Statice*s, which are so well represented at Blakeney, present a series of species which, like the *Salicornias*, occupy successively higher zones of the marsh. Of these *Statice humilis* occupies the most frequently inundated marshes, and it may be for this reason is the only British species which fails to grow vigorously in ordinary garden soil. *Suaeda fruticosa* and *Statice binervosa* will, on the other hand, grow in garden soil with extreme luxuriance.

Of the *Salicornietum* samples, those from the middle region of the *Pelvetia* marsh represent an old condition, whilst those from the Samphire marsh represent a very recent one. It will be noted that the proportion of organic material is greater in the older marsh. Similarly, whilst the relatively recent *Obione* zone of the *Pelvetia* marsh occupies a soil with from 1.01 to 5.21 per cent. organic material, that of the old *Obione* marshes

ranges from 12.64 to 21.69 per cent. There is evidently then a tendency for the organic content to increase with the lapse of time.

SUMMARY.

An account is given of the soils of Blakeney Point as they affect the vegetation, based on the examination of a large number of samples from phases of very diverse age, of which, however, the sequence is known with a high degree of certainty.

These show that the dune systems as they grow older exhibit a diminution of carbonates and an increase of the organic content. Accompanying these changes, which are the result of leaching and the augmented plant covering, there is a change from an appreciably alkaline condition exhibited by the embryo dunes to a marked acidity in the oldest phases. With these edaphic changes are correlated the accompanying succession in the vegetation.

The part played by rabbit droppings in influencing the organic content and the reaction is considered, and quantitative data are furnished.

The hydrogen-ion concentration is shown to vary, not only with the degree of leaching and organic content, but also according to the source of origin of the organic material and the phase of its decomposition.

The relation between the organic content and the water-content of dune soils is shown to be a close one.

The shingle banks are found to show a similar sequence alike in respect to reaction, organic content, and water-content, as also in stability. This is brought out by a study of shingle laterals of successive age, of which also tabulated floristic lists are given.

The salt-marsh phases likewise show indications of increasing organic content, but here the important edaphic factor would appear to be the duration and frequency of tidal inundation.

APPENDIX.

TABLE I. *Dune Soils.*

Sample No.	Young Dunes J. and G. Series.	p. H.	Loss on ignition. %	Total carbonates. %
J. 030	Embryo <i>Psamma</i> dune E. of Lifeboat House	7.2	0.37	0.34
J. 031	" " W. " "	7.3	0.35	0.39
J. 022	" " " " "	7.1	—	—
J. 023	" " " " "	7.1	—	—
J. 024	Rather larger than 022-023 same system	7.0	—	—
J. 032	Very young <i>Psamma</i> dune on New Spit	7.4	0.16	0.44
J. 033	" <i>Triticum</i> " "	7.3	0.43	0.46
J. 036	" " " "	7.4	0.34	0.32
G. 01	" dune on sea-face	7.4	0.52	0.35
G. 001	" " "	7.4	—	0.39
G. 014	" dune 4 in. high	7.2	—	—
G. 013	" dune 12 in. high near 014	7.1	—	—
G. 015	Young dune near 013, 30 in. high (0-4)	7.0	0.30	0.61
G. 016	Same as 015 (4-10)	6.9	—	0.41
G. 025	Very young outer dune	7.2	0.39	0.36

TABLE I (continued).

Sample No.	Young Dunes J. and G. Series.	p. H.	Loss on ignition. %	Total carbonates. %
G. 026	Very young outer dune	7.4	—	0.58
G. 09	" " 30 yds. seawards of 010	7.1	—	—
G. 010	Young dune larger than 09 on sea-face	7.0	—	0.58
G. 017	Near but larger than 016, 0-4 in.	7.05	—	—
G. 018	As 017, but 4-10 in.	6.9	—	—
G. 07	Young dune 50 yds. SW. of 010	7.1	0.33	0.32
G. 071	" " " "	7.1	—	0.39
G. 05	50 yds. SW. of 07	7.2	—	—
G. 06	13 yds. landward of 05	7.2	—	—
G. 08	13 yds. " 07	7.1	—	—
G. 027	Very young dune near 'Glaux' low	7.1	0.23	0.38
G. 028	" " " "	—	0.35	0.35
G. 029	" " " "	—	—	0.28
F. 019	Hollow between young dunes	7.0	—	—
F. 02	Young dune, <i>Psamma</i> flowering freely	7.3	—	—
F. 03	" 75 yds. NE. of 01	7.3	0.46	0.60
F. 04	Larger dunes of outer system 17 yds. N. of 03	7.1	—	—
F. 011	Outermost dunes with <i>Psamma</i> and <i>Arenaria</i> <i>peploides</i> 0-4 in.	7.1	0.36	0.34
F. 012	As 011 but 4-10 in.	7.1	—	0.57
F. 020	Older dune 0-4 in.	7.0	0.42	0.36
F. 021	As 020 but 4-10 in.	7.1	0.42	0.41

p. H. range, 6.9-7.4; av. 7.1. Loss on ignition, 0.23-0.52 %; av. 0.36 %. Carbonates, 0.28-0.61 %; av. 0.425.

Main Ridge. Series E.

E. 1	Seaward slope near 'Glaux' low 0-4 in.	6.9	—	—
E. 2	" " " 4-8 in.	6.9	—	—
E. 3	Landward slope 13 yds. from E. 1	7.0	—	—
E. 4	Near big 'blow-out'	7.0	—	—
E. 5	5 yds. S. of Notice Board	6.9	—	—
E. 8	20 yds. SW. of E. 7 in hollow 0-4 in.	7.1	—	—
E. 9	Same as E. 8 in hollow 4-8 in.	6.9	—	0.65
E. 12	Burnt patch of <i>Psamma</i> seaward slope	6.9	—	0.47
E. 13	" " landward slope	7.0	—	—
E. 16	8 yds. down seaward slope near E. 14	7.0	0.34	0.43
E. 17	8 yds. " landward " "	6.9	—	—
E. 20	Hollow 25 yds. S. of E. 18 0-4 in.	6.9	0.33	0.21
E. 21	" " " 4-8 in.	7.1	—	0.23
E. 22	Southern edge of main ridge	7.0	—	—
E. 24	Seaward slope	7.3	—	0.65
E. 25	Slope	—	0.29	0.21
E. 32	" "	—	—	0.20
E. 33	" "	—	1.60	0.15
E. 6	Crest of ridge near E. 8	7.1	0.38	0.23
E. 7	" " " E. 6	7.0	0.51	0.50
E. 10	" " " "	7.0	—	—
E. 11	" " " "	6.9	0.39	0.41
E. 14	0-4 in.	7.0	—	—
E. 15	4-10 in.	7.1	—	—
E. 18	Crest extreme outer end of ridge 0-4 in.	6.9	—	—
E. 19	" " " 4-10 in.	7.0	—	—
E. 23	Crest of main ridge	7.1	0.40	0.32
E. 26	" "	7.2	0.51	0.49
E. 27	" "	7.2	0.69	0.20
E. 28	" "	7.1	0.64	0.27
E. 29	" "	7.2	0.40	0.26
E. 30	" "	7.1	0.38	0.20
E. 31	" "	7.2	0.24	0.40

p. H., 6.9-7.3; av. 7.03. Loss on ignition, 0.24-1.60 %; av. 0.501 %. Carbonates, 0.2-0.65 %; av. 0.341.

TABLE I (continued).

Laboratory. Ridge D.

Sample No.	Young Dunes J. and G. Series.	p. H.	Loss on ignition. %	Total carbonates. %
D. 1	<i>Tortula ruraliformis</i> and <i>Cladonia</i>	6.9	0.56	0.05
D. 2	Highest part of	6.9	—	—
D. 3	" "	6.9	—	—
D. 4	" "	6.8	—	—
D. 5	" "	6.9	—	—
D. 6	" "	6.8	—	—
D. 7	122 yds. along ridge D	6.95	0.46	0.15
D. 8	<i>Psamma</i> dom.	6.80	0.39	0.64
D. 12	Side of 'blow-out', <i>Psamma</i> dom. 0-4 in.	6.8	0.30	0.34
D. 11	" " " 12 in.	6.9	—	—
D. 10	" " " 24 in.	6.95	—	—
D. 9	" " " 36 in.	6.9	—	—
D. 13	Ridge D. near Lifeboat House 0-4 in.	6.9	0.40	0.09
D. 14	" " " 4-8 in.	7.0	—	0.12
D. 15	<i>Tortula-Psamma</i> near Lab. 0-4 in.	6.9	—	—
D. 16	" " " 4-8 in.	6.9	—	—
D. 17	" another locality 0-4 in.	6.9	0.53	0.05
D. 18	" " 4-8 in.	7.1	0.35	0.05
D. 19	Crest, 0-4 in.	6.9	—	—
D. 39	<i>Tortula</i> dom., <i>Cladonia</i>	—	0.58	0.03
D. 40	<i>Brachythecium albicans</i>	7.0	0.44	0.11
D. 36	<i>Tortula ruraliformis</i>	7.3	0.67	—
D. 37	" "	7.0	0.69	0.16
D. 38	" "	7.0	0.70	—
D. 44	Dense <i>Cladonia</i> and <i>Hypnum</i> 0-1 in.	5.6	—	—
D. 45	" " " 1-6 in.	6.7	—	—
D. 43	<i>Brachythecium albicans</i> dom.	—	0.76	0.07

p. H., 5.6-7.1; av., 6.908. Loss on ignition, 0.3-0.76 %; av., 0.525 %. Carbonates, 0.3-0.64 %; av., 0.155 %.

Ridge C.

C. 20	<i>Cladonia</i> dom. 0-1 in.	6.8	—	0.00
C. 21	" " 3 in.	6.9	—	—
C. 22	" " 6 in.	7.0	—	—
C. 23	" " 9 in.	7.0	—	—
C. 24	" near C. 20 0-1 in.	6.0	—	—
C. 25	" dom. 3 in.	6.9	—	—
C. 26	" " 6 in.	6.9	—	—
C. 27	" " 9 in.	6.9	—	—
C. 28	" " 0-1 in.	6.4	—	—
C. 29	" " 3 in.	6.9	—	—
C. 30	" " 6 in.	6.9	—	—
C. 31	" " 9 in.	6.9	—	—
C. 32	" " 0-1 in.	6.9	—	—
C. 33	" " 3 in.	7.1	—	—
C. 34	" " 6 in.	7.1	—	—
C. 35	" " 9 in.	7.1	—	—
C. 40	" " 0-4 in.	6.8	—	0.00
C. 41	" " 0-4 in.	—	0.82	0.05
C. 42	" " 0-4 in.	—	0.90	0.07
C. 46	<i>Soldanella</i> dune with freshly-blown sand	7.1	—	0.33
C. 47	" " " "	7.1	—	0.37

p. H., 6.0-7.1; av. 6.89; av. organic, 0.86 %. Carbonates, 0.00-0.37 %; av. carbonates (4 samples excluding C. 46 and C. 47 where accretion occurs), 0.03 %.

TABLE I (continued).

Long Hills. Series B.

Sample No.	Young Dunes J. and G. Series.	p. H.	Loss on ignition. %	Total carbonates. %
B. 1	Seaward slope near main beach, some accretion	7.0	0.56	0.75
B. 2	Middle of dune in line with B. 1 and B. 3	6.0	1.10	0.00
B. 3	Landward slope	6.5	1.32	0.05
B. 4	Crest between B. 2 and B. 5	6.0	—	—
B. 5	<i>Silene maritima</i> dom.	6.7	0.69	0.00
B. 6	<i>Polypodium vulgare</i> patch 0-4 in.	5.9	2.69	0.00
B. 7	" " 4-8 in.	6.0	0.66	0.00
B. 8	8 yds. S. of B. 16 0-4 in.	6.0	0.64	0.00
B. 9	" " 4-8 in.	6.0	—	0.05
B. 10	25 yds. E. of bow of 'Yankee'	6.1	—	0.00
B. 11	Landward slope, middle 0-4 in.	6.1	—	—
B. 12	" " 4-8 in.	6.7	—	—
B. 13	Crest near B. 11 0-4 in.	6.5	1.03	0.00
B. 14	" " 4-8 in.	6.8	—	0.02
B. 15	Seaward slope near B. 11 0-4 in.	6.9	—	—
B. 16	" " 4-8 in.	6.9	—	—
B. 17	Seaward slope 0-4 in.	6.9	1.50	0.00
B. 18	Crest 0-4 in.	6.0	—	0.01
B. 19	Landward slope near B. 18 0-4 in.	6.8	—	—
B. 20	" " 4-8 in.	6.7	—	—
B. 21	Crest " " 0-4 in.	—	1.30	—
B. 22	" " 0-4 in.	—	1.21	—

p. H., 5.9-7.0; av., 6.38. Loss on ignition, 0.56-2.69 %; av., 1.154 %. Carbonates, 0.00-0.75 %; av. carbonates (exclusive of B. 1) = 0.01 %.

The Hood. Series A.

A. 1	Hollow with sparse <i>Psamma</i> and <i>Carex arenaria</i> c. 0-2 in.	6.1	10.10	0.00
A. 2	Hollow with sparse <i>Psamma</i> and <i>Carex arenaria</i> c. 2-4 in.	6.2	1.04	0.00
A. 3	<i>Corynephorus canescens</i> dom. (seaward face)	6.6	3.20	0.02
A. 4	Sparse <i>Psamma</i> mosses and lichens	6.7	—	0.05
A. 5	" " " "	—	1.90	—
A. 6	Crest W. side, some new-blown sand	6.9	0.61	0.03
A. 7	Centre of hollow, mosses about	6.3	3.00	0.00
A. 8	Dense vegetation	6.0	6.34	0.01
A. 9	W. of summit	6.3	1.34	0.00
A. 10	E. " "	6.1	2.76	0.01
A. 11	Outlying dune near Hood	6.3	1.41	0.02
A. 12	N. side of crest, <i>Carex arenaria</i> dom.	6.1	1.93	0.00
A. 13	NE. corner	5.5	3.51	0.00
A. 14	Dense vegetation	—	2.57	—
A. 15	Crest	—	1.85	—
A. 16	" "	—	1.60	—

p. H., 5.5-6.9; av., 6.24. Loss on ignition (0-4 in.), 0.61-6.34 %; av., 2.69 %. Carbonates, 0.00 to 0.05 %; av., 0.01 %.

TABLE XI.

Lateral Shingle Hooks.

<i>Sample No.</i>	<i>Bank No.</i>	<i>Location on lateral.</i>	<i>Vegetation.</i>	<i>p. H.</i>	<i>Loss on ignition.</i>	<i>Total carbonates.</i>
					%	%
S. 16	XX	Crest	Bare shingle and sand	7.6	0.77	0.96
S. 17	"	"	" " "	7.6	0.65	0.51
S. 18	"	"	" " "	7.5	0.19	0.82
S. 19	"	"	" " "	7.6	0.57	0.87
S. 20	"	"	" " "	7.6	0.44	0.50
S. 21	"	"	" " "	7.6	0.30	1.22

Av. organic, 0.486 %; range, 0.19-0.77 %. Carbonates av., 0.813 %; range, 0.50-1.22 %.

X. 23	XVII	Crest	Bare shingle close to Britannia	7.5	0.53	0.35
X. 20	"	"	<i>Arenaria peploides</i> patch due S. of Britannia	7.1	0.32	0.05
X. 19	"	"	<i>Arenaria peploides</i> , <i>Silene maritima</i>	7.1	—	0.08
X. 25	"	"	Crest above X. 24	7.2	0.13	0.11
X. 24	"	Slope	Slightly sandy, with <i>P. coronopus</i> , <i>f. pygmaea</i>	7.1	—	0.05
X. 21	"	Drift line	On landward drift line N. of X. 20	7.2	—	—
X. 22	"	" "	On seaward drift line S. of X. 20	7.05	—	—

Av. p. H., 7.18 %; range, 7.05-7.5 %. Carbonates av., 0.16 %. Organic av., 0.32 %.

X. 26	XVI	Crest	<i>Arenaria peploides</i> , <i>Silene</i>	7.0	—	—
X. 28	XV	Base of slope	<i>Stachys fruticosa</i> zone	7.3	—	—
X. 27	"	Upper part of slope	<i>Stachys binervosa</i> zone	7.15	—	—
X. 41	XII	Crest	Proximal end, SW. of 'Yankee'	7.1	1.8	0.03
X. 41 ^a	"	"	"	7.1	1.79	—
X. 42	"	"	Middle. 80 yds. S.W. of 'Yankee'	7.2	7.12	0.06
X. 43	"	"	Distal end, 200 yds. SW. of 'Yankee'	6.8	22.42	0.16
X. 29	"	"	Turfy patch, <i>Sedum</i> , <i>P. coronopus</i> , <i>Cladonia</i>	6.3	6.02	0.09
X. 33	"	"	Very firm shingle, moss and <i>Cladonia</i>	6.2	—	—
X. 35	"	"	Very firm shingle, lichens about 25 yds. S.W. of 'Yankee'	6.7	3.57	0.01
X. 36	"	"	Open veg. 3 yds. from X. 35	6.7	—	—
X. 34	"	"	Loose shingle near X. 33	6.3	—	—
X. 30	"	"	" " X. 29	6.8	—	—
X. 6	"	<i>Binervosa</i> zone	Dense <i>Stachys binervosa</i>	7.0	0.47	0.01
X. 31	"	Drift line	<i>Armeria</i> and <i>Silene</i>	7.0	2.99	0.27

Av. p. H., 6.72 (range, 6.2-7.2). Av. organic, 7.12 % (range, 1.79-22.46 %). Carbonates av., 0.1031%.

X. 44	VIII	Crest	Turf vegetation, proximal end	6.75	6.45	0.04
X. 45	"	"	" " middle	6.0	11.90	0.03
X. 46	"	"	High elbow, distal end	5.9	25.06	0.01
X. 46 ^a	"	"	" " "	—	28.90	0.00
X. 17	"	"	<i>Cladonia, Armeria, Silene</i>	6.9	3.88	0.07
X. 18	"	Drift zone	<i>Atriplex littoralis, Triticum</i>	7.6	16.14	3.55
X. 37	"	" "	" " "	—	—	4.20
X. 40	"	" "	" " "	—	41.5	—
X. 38	"	Slope	<i>Cochlearia danica</i> and <i>Triticum</i>	7.3	—	0.14
X. 16	"	<i>Binervosa</i> zone	<i>Statice binervosa</i>	7.35	2.09	0.01
X. 39	"	" "	" " and <i>Armeria</i>	7.1	—	0.07

p. H., 5.9-7.6; av., 6.87. Organic, 2.09-41.5 %; av., 16.99 %. Carbonates, 0.00-4.2 %; av., 0.112 %.

TABLE XI (continued).

Sample No.	Bank No.	Location on lateral.	Vegetation.	p. H.	Loss on ignition. %	Total carbonates. %
X. 47	VII	Crest	Turfy, proximal end	7.0	3.28	0.02
X. 48	"	"	" middle	6.9	2.64	0.03
X. 48 ^a	"	"	Middle	—	2.71	—
X. 49	"	"	Distal end	6.6	4.31	0.03
X. 1	"	"	High elbow, <i>Aira praecox</i> , &c.	6.0	14.68	0.04
X. 61	"	"	" " <i>Cladonia</i> , <i>Armeria</i> ,	—	7.01	—
X. 2	"	"	" " <i>Silene</i>	6.0	—	—
X. 62	"	"	<i>Cladonia</i> , &c.	—	4.04	—
X. 5	"	" 0-2 in.	<i>Festuca</i> , <i>Triticum</i> , <i>Cladonia</i>	6.7	—	—
X. 6	"	" 2-4 "	" " "	6.9	—	—
X. 4	"	<i>Binervosa</i> zone	<i>Statice binervosa</i> and <i>Franke-</i> <i>nia</i> , W. side	7.3	—	—
X. 7	"	" "	<i>Statice binervosa</i> and <i>Franke-</i> <i>nia</i> , E. side	7.5	3.28	0.04
X. 59	"	" "	<i>Statice binervosa</i> and <i>Franke-</i> <i>nia</i> , E. side	—	2.43	—
X. 8	"	<i>Suaeda</i> zone	<i>Suaeda fruticosa</i> , W. side	7.4	15.70	2.55
X. 60	"	" "	" "	—	15.50	—
X. 3	"	" "	<i>Suaeda</i> , <i>Artemisia</i> , <i>Festuca</i> , E. side	7.2	4.9	—
p. H., 6-7.5; av. crest, 6.59. Organic (crest), 2.64-14.68 %; av., 5.52 %. Carbonates (crest), 0.02-0.04 %; av., 0.03 %.						
X. 50	IV	Crest	Proximal end	6.95	2.12	0.00
X. 51	"	"	Middle of bank	6.9	2.52	0.01
X. 51 ^a	"	"	" "	7.05	—	—
X. 52	"	"	Distal end	6.9	2.55	0.00
X. 52 ^a	"	"	" "	7.0	—	—
Av. p. H., 6.96. Av. organic, 2.39 %. Carbonates av., 0.00 %.						
X. 53	III	Crest	Proximal end	6.9	3.23	0.01
X. 54	"	"	Middle	6.85	1.82	0.01
X. 55	"	"	Distal end	6.85	2.89	0.00
Av. p. H., 6.86. Av. organic, 2.64 %. Carbonates av., 0.006 %.						
X. 56	I	Crest	Proximal end	7.1	3.82	0.03
X. 57	"	"	Middle, <i>Cladonia</i> , <i>Silene</i>	7.1	3.78	0.03
X. 57 ^a	"	"	" " "	7.1	2.18	—
X. 58	"	"	Distal end	6.8	2.90	0.00
X. 15	"	"	<i>Silene maritima</i> , <i>Cladonia</i>	6.9	—	—
X. 9	"	"	<i>Agrostis maritima</i> , <i>Cladonia</i> , 30 yds. from high elbow	6.9	3.31	0.00
X. 13	"	"	<i>Silene</i> , <i>Festuca</i> , <i>Aira</i> , mosses	6.5	—	—
X. 14	"	"	" " " " "	6.8	—	—
10 yds. E. of X. 13						
X. 10	"	<i>Binervosa</i> zone	<i>Statice binervosa</i> , <i>Franckenia</i>	7.1	4.91	0.01
X. 10 ^a	"	" "	" " "	7.1	—	0.03
X. 11	"	Drift zone	<i>Triticum</i> , <i>Festuca</i> , <i>Atriplex</i> ,	7.0	—	—
0-2 in.						
X. 12	"	" "	<i>Triticum</i> , <i>Festuca</i> , <i>Atriplex</i> ,	7.3	6.38	0.04
2-4 in.						

Crest, p. H., 6.5-7.1; av., 6.90. Crest, organic, 2.18-3.82 %; av., 3.19 %. Carbonates, 0.00-0.03 %.

TABLE XIII.

Main Beach.

Sample No.	Location on lateral.	Vegetation.	p. H.	Loss on	Total
				ignition.	carbonates.
				%	%
S. 1	Crest	<i>Arenaria peploides</i> , sparse	7.0	—	—
S. 2	"	" " dense	7.3	0.40	0.39
S. 15	"	" " " under mat	7.2	—	—
S. 16	"	" " " "	7.3	—	—
S. 8	"	Shingle with <i>Silene maritima</i>	7.1	—	—
S. 13	"	Beneath mat of <i>S. maritima</i>	7.2	—	—
S. 14	"	" " "	7.3	5.08	1.20
S. 11	"	" rosette of <i>Rumex trigranulatus</i>	6.9	—	2.10
S. 12	"	Beneath rosette of <i>Rumex trigranulatus</i>	6.7	—	1.90
S. 10	"	Beneath bush of <i>Suaeda fruticosa</i> just below crest	7.0	—	—
S. 9	"	Beneath bush of <i>Suaeda fruticosa</i> just on crest	6.9	6.49	1.36
S. 30	"	Beneath bush of <i>Suaeda fruticosa</i>	—	2.00	—
S. 31	"	" " " "	—	3.30	—
S. 32	"	" " " "	—	2.1	—
S. 33	"	" " " "	—	1.4	—
S. 34	"	" " " "	—	2.0	—
S. 35	"	Fine shingle, shallow-rooted grasses, <i>Lepturus</i> , &c.	—	0.0	—
S. 6	"	Fine shingle with <i>S. binervosa</i> and <i>Sclerochloa loliacea</i>	7.1	—	—
S. 36	"	Bare fine shingle	—	0.0	—
S. 3	"	Fine shingle, bare of vegetation	7.1	0.39	0.83
S. 4	"	" " " " near embryo dunes	7.25	—	—
S. 7	"	Bare shingle, landward side of dunes	7.2	0.69	0.23
S. 36	"	Bare shingle	—	1.0	—
S. 37	"	" close to <i>Suaeda</i> bush	—	1.6	—
S. 38	"	" " "	—	1.5	—
S. 39	"	" " "	—	1.1	—
S. 40	"	" " "	—	1.3	—
S. 41	"	Bare shingle	—	1.1	—
S. 42	"	"	—	1.3	—
S. 43	"	"	—	0.9	—

Main beach, p. H., 6.7-7.3; av., 7.11. Organic, 0.0-6.49 %; av., 1.68 %. Carbonates, 0.23-2.1 %; av., 1.14 %.

Shingle 'Lows'.

	Vegetation.	p. H.	Loss on ignition.
			%
1	0-4 in. 'long' low. <i>Plantago coronopus</i> , <i>f. pygmaea</i>	6.9	0.79
2	" " " "	6.9	—
3	" " " "	6.9	—
4	" " " "	7.0	—
5	4-8 in. " " "	6.9	—
6	" " " "	6.9	—
7	" " " "	6.9	—
8	'Glaux' low	—	0.52
9	Low near Lifeboat House, <i>S. reticulata</i> decreasing	—	0.81
10	Low at Hood, <i>P. coronopus</i> , <i>f. pygmaea</i> , dead <i>Suaeda</i>	—	1.31
11	Terminal low, 'Long Hills'	—	1.75
12	Small low, centre of 'Long Hills'	—	1.22
13	Large " " "	—	3.3

TABLE XIV. *Vegetation of Lateral Banks.*

(For numbering see map, Text-fig. 1.)

<i>Species.</i>	Youngest ← → Oldest							
	XX.	XVII.	XII.	VIII.	VII.	VI.	IV.	I.
<i>Agrostis maritima</i>	—	—	—	c.	c.	c.	c.	c.
<i>Aira praecox</i>	—	—	f.	f.	l.c. (high elbow)	r.	—	—
<i>Anagallis arvensis</i>	—	—	o.-f.	—	—	—	—	—
<i>Arenaria peploides</i>	o.	c.	c.	l.	l.	—	—	—
<i>serpyllifolia</i>	—	—	—	c.	c.	c.	c.	—
<i>Ameria maritima</i>	—	—	v.c.	v.c.	v.c.	c.	c.	f.c.
<i>Arrhenatherum elatius</i>	—	—	—	v.r.	—	—	—	—
<i>Artemisia maritima</i>	—	o.	f.	c.	c.	c.	c.	c.
<i>Atriplex babingtonii</i>	—	—	f.	f.	—	—	—	—
<i>littoralis</i>	—	—	o.	l.f.	—	—	—	—
<i>Bellis perennis</i>	—	—	—	r.	—	—	—	—
<i>Bromus mollis</i>	—	—	v.r.	f.	l.f. (high elbow)	—	—	r.
<i>Cakile maritima</i>	f.	—	—	—	—	—	—	—
<i>Cerastium semidecandrum</i>	—	—	—	f.	f.c.	—	—	—
<i>tetrandrum</i>	—	—	f.c.	o.	r.	r.	f.	f.
<i>Cirsium lanceolatum</i>	—	—	—	r.r.	—	—	—	—
<i>Cochlearia danica</i>	—	—	r.	l.f.	l.f.	r.	—	r.r.
<i>officinalis</i>	—	—	l.f.	l.f.	—	—	—	—
<i>Crepis capillaris</i>	—	—	—	v.r.	—	—	—	—
<i>Erodium neglectum</i>	—	—	o.	r.	—	—	—	—
<i>Festuca myuros</i>	—	—	—	v.r.	—	—	—	—
<i>ovina</i>	—	—	—	l.	—	v.r.	—	—
<i>rubra</i>	—	—	—	f.	c.	c.	c.	c.
<i>Filago minima</i>	—	—	r.	—	—	—	—	—
<i>Frankenia laevis</i>	—	—	l.c.	c.	c.	c.	c.	f.
<i>Galium verum</i>	—	—	—	f. (high elbow)	r. (high elbow)	—	—	—
<i>Geranium molle</i>	—	—	v.r.	v.r.	—	—	—	—
<i>Glaucium luteum</i>	v.r.	v.r.	—	—	—	—	—	—
<i>Glyceria maritima</i>	—	—	—	c.	c.	c.	c.	c.
<i>Hordeum murinum</i>	—	—	—	r. (high elbow)	—	—	—	—
<i>Hypochoeris glabra</i>	—	—	r.	—	—	—	—	—
<i>Koeleria cristata</i>	—	—	—	r.	—	—	—	—
<i>Lepturus filiformis</i>	—	—	r.	l.	r.r.	l.f.	l.f.	f.
<i>Lotus corniculatus</i>	—	—	f.	f.c.	f.c.	l.	—	f.
<i>Myosotis collina</i>	—	—	v.r.	—	f.	—	—	—
<i>Obione portulacoides</i>	—	—	o.	f.	l.f.	l.f.	f.	f.
<i>Phleum arenarium</i>	—	—	v.r.	—	—	—	—	—
<i>Plantago coronopus</i>	—	o.	f.c.	f.c.	o.-l.f.	l.f.	f.	f.c.
<i>lanceolata</i>	—	—	—	f.	l.f. (high elbow)	—	—	—
<i>Poa annua</i>	—	—	f.	o.	—	—	—	—
<i>loliacea</i>	—	—	o.	—	r.r.	—	l.f.	l.f.
<i>pratensis</i>	—	—	—	f.c.	f.	l.f.	r.	r.r.
<i>Psamma arenaria</i>	o.	—	r.	—	—	—	—	—
<i>Rumex acetosella</i>	—	—	f.	f.c.	f.	—	l.f.	f.
<i>crispus v. trigranulatus</i>	—	—	f.	o.	r.r.	v.r.	—	—
<i>Sagina maritima</i>	—	—	o.	o.	r.r.	r.	—	l.f.
<i>Salsola kali</i>	f.	—	—	—	—	—	—	—
<i>Sedum acre</i>	—	—	f.	f.	f.c.	f.	f.	f.
<i>anglicum</i>	—	—	—	o. (high elbow)	r. (high elbow)	—	—	—
<i>Senecio jacobaea</i>	—	—	f.	v.r.	v.r.	—	—	—
<i>sylvaticus</i>	—	—	—	r.	—	—	—	—
<i>Silene maritima</i>	—	ab.	ab.	v.c.	c.	c.	c.	f.c.
<i>Spergularia salina</i>	—	—	—	v.r.	—	—	—	—
<i>Statice binervosa</i>	—	—	c.	c.	c.	c.	c.	c.
<i>Stellaria boreana</i>	—	—	v.r.	—	—	—	—	—

TABLE XIV (continued).

Species.	Youngest ← —————→ Oldest.									
	XX.	XVII.	XII.	VIII.	VII.	VI.	IV.	III.	I.	
<i>Suaeda fruticosa</i>	—	c.	c.	c.	c.	c.	c.	c.	v.c.	
<i>Trifolium arvense</i>	—	—	—	f.	l.f.	r.	r.	f.	r.	
„ <i>procumbens</i>	—	—	—	r. (high elbow)	v.r. (high elbow)	—	—	—	—	
„ <i>striatum</i>	—	—	—	l.f.	—	—	—	—	—	
<i>Triticum junceum</i>	o.	—	—	f.	c.	l.	l.c.	l.c.	—	
„ <i>pungens</i>	—	—	—	l.c.	l.c.	c.	c.	c.	c.	
<i>Vicia angustifolia</i>	—	—	—	f. (high elbow)	v.r. (high elbow)	—	—	—	—	
Totals	6	6	34	51	36	25	21	24	16	

ab. = abundant; c. = common; f. = frequent; o. = occasional; l. = local; r. = rare, &c.

TABLE XV.

Sample No.	Marsh soils.	p. H.	Loss on ignition.	Carbonates.
			%	%
K. 7	<i>Salicornietum</i> (<i>S. herbacea</i>)	7.3	—	—
K. 8	„ „ „ <i>Pelvetia</i>	7.6	—	—
K. 9	Bare area near K. 8	7.3	1.08	1.72
K. 15	<i>S. herbacea</i> , <i>Pelvetia</i> , <i>Obione</i> sparse	7.3	—	—
K. 16	„ „ „	7.6	—	—
K. 17	„ „ „ and <i>Aster tripolium</i>	7.6	—	—
K. 29	Same transect as K. 27 and K. 28	7.5	—	—
K. 32	„ „ „ K. 30 „ K. 31	7.5	9.26	2.26
K. 35	„ „ „ K. 33 „ K. 34	7.4	—	—
K. 38	„ „ „ K. 36 „ K. 37	7.5	—	—
K. 40	<i>S. herbacea</i> , <i>Pelvetia</i> , <i>Pelvetia</i> marsh	—	—	0.14
K. 46	Same transect as K. 47 and K. 41 (near edge)	7.6	5.33	2.18
K. 44	Same transect as K. 43 and K. 48 (near edge)	7.5	4.78	1.75
K. 45	Same transect as K. 42 and K. 49 (near edge)	7.5	4.71	2.70
K. 55	<i>Pelvetia</i> marsh E. (middle)	8.0	14.63	2.02
K. 56	„ „ „ centre „	8.0	14.41	2.04
K. 57	„ „ „ west „	8.2	12.18	3.50
K. 52	Samphire marsh E.	7.9	12.20	5.10
K. 53	„ „ „ middle	8.0	11.13	4.55
K. 54	„ „ „ west	7.8	11.36	2.95

p. H. (7.3-8.2); av., 7.63. Loss on ignition, 1.08-14.63 %; av., 9.19 %. Carbonates, 0.14-5.10 %; av., 2.55 %.

K. 5	Centre of <i>Obione</i> zone	7.3	1.01	0.62
K. 4	Upper edge „	7.3	—	—
K. 6	Lower edge „	7.1	—	—
K. 13	Another transect, centre of <i>Obione</i> zone	7.3	—	0.20
K. 12	Upper edge „ „	7.25	—	—
K. 14	Lower edge (with <i>S. radicans</i>)	7.2	—	—
K. 28	Centre of <i>Obione</i> zone (cf. K. 27 and K. 29)	7.6	—	—
K. 31	„ „ „ (cf. K. 30 and K. 32)	7.6	3.21	1.83
K. 34	„ „ „ (cf. K. 33 and K. 35)	7.4	—	—
K. 37	„ „ „ (cf. K. 36 and K. 38)	7.4	—	—
K. 21	„ 0-2 in.	7.5	—	—
K. 22	„ 8-10 in.	7.15	—	—
K. 23	„ 15-18 in.	7.2	—	—
K. 39	„ of <i>Obione</i> zone	—	—	1.00
K. 41	<i>Obione</i> zone	7.4	5.21	1.55
K. 48	„	7.45	2.46	0.55
K. 49	„	7.4	1.84	0.70
K. 50	Old <i>Obione</i> salting	—	2.40	—

p. H., 7.1-7.6; av., 7.34. Loss on ignition, 1.01-21.40 %; av. (exclusive of K. 50), 2.74 %. Carbonates, 0.55-1.83 %; av., 0.92 %.

TABLE XV (continued).

Suaeda Zone.

Sample No.	Marsh soils.	p. H.	Loss on ignition. %	Carbonates. %
K. 1	Centre of <i>Suaeda fruticosa</i> zone	7.3	1.77	0.71
K. 2	Upper edge	7.1	—	—
K. 3	Lower "	7.2	—	—
K. 10	<i>Suaeda</i> zone on sand	7.6	—	—
K. 11	Slightly higher than K. 11	7.3	—	—
K. 27	Centre of zone (cf. K. 28 and K. 29)	7.3	—	—
K. 30	" " (cf. K. 31 and K. 32)	7.6	1.70	1.49
K. 33	" " (cf. K. 34 and K. 35)	7.6	—	—
K. 36	" " (cf. K. 37 and K. 38)	7.4	—	—
K. 18	0-2 in.	7.35	—	—
K. 19	8-12 in. (maximum root zone)	7.2	—	—
K. 20	18-20 in.	7.3	—	—
K. 42	Centre of zone, same transect as K. 45 and K. 49	7.5	0.59	0.26
K. 43	Centre of zone, same transect as K. 44 and K. 48	7.4	1.33	0.57
K. 47	Centre of zone, same transect as K. 41 and K. 46	7.5	1.41	0.35

p. H., 7.1-7.6 (av., 7.37). Loss on ignition, 0.59-1.77 %; av., 1.36 %. Carbonates, 0.26-1.49 %; av., 0.676 %.

TABLE XVI.

Percentage Chlorides taken from the Unpublished Observations of Blakeney Point (Estimations by Miss A. C. Halket and Dr. H. B. Hutchinson),

Soil type.	Vegetation.	Chlorides. %
Sand dune, main ridge	<i>Psamma, Arenaria</i>	0.0042-0.0046
Dune, Long Hills	0-6 in. <i>Psamma</i> , &c.	0.010
"	6-12 in. "	0.014
"	12-18 in. "	0.005
"	0-6 in. bare sand	0.000
"	6-12 in. "	0.01
"	12-18 in. "	0.198
Main shingle bank	Sparse <i>Arenaria</i> and <i>Silene</i>	0.0017
"	Bare shingle	0.25
"	"	1.17
"	"	0.86
"	"	0.33
"	"	0.26
"	"	0.98
"	"	0.92
"	"	0.44
"	Under <i>Suaeda fruticosa</i>	1.72
"	" "	3.08
"	" "	1.30
"	" "	0.85
"	" "	2.10
Gully on main bank (fine shingle)	<i>Desmazeria loliacea</i> and <i>Lepturus filiformis</i>	0.12
Side of gully	Bare	0.26

Range
0.25-1.17
Av. 0.65

0.85-3.08
Av. 1.81

TABLE XVI (continued).

Soil type.		Chlorides.
		%
<i>Lateral shingle bank—</i>		
High elbow, bank 7		0.29
Crest, bank 7		0.17
" " 8		0.072
<i>Statice binervosa</i> zone, bank 7		0.90
" " " 7		0.005
<i>Triticum</i> zone, bank 8		0.158
<i>Festuca</i> zone, bank 7		1.60
<i>Suaeda fruticosa</i> zone, bank 7		3.05
" " " 8		0.197
" " " 7		0.4633
" " " 7		0.3941
" " main beach (0-9)		0.232
" " (0-9)		0.3536
" " (9-18)		0.3378
" " (18-24)		0.2802
Locality.		Chlorides.
		%
<i>Shingle lozes—</i>		
End of Long Hills	<i>Statice reticulata</i> , <i>Frankenia laevis</i> , <i>Plantago coronopus</i>	0.36
Large low middle Long Hills	<i>Statice reticulata</i> , <i>Frankenia laevis</i> , <i>Plantago coronopus</i>	3.50
Small " "	<i>Statice reticulata</i> , <i>Frankenia laevis</i> , <i>Plantago coronopus</i>	0.30
Low W. Lifeboat House	<i>S. reticulata</i> , dying out	1.04
Low on Hood	Dead <i>Suaeda</i> bushes	1.39
'Glaux' Lagoon	<i>Glaux maritima</i>	1.70
<i>Salt marsh—</i>		
<i>Obione</i> zone	<i>Obione</i> , <i>Salicornia</i>	0.55
" " "	" " "	1.17
<i>Salicornia radicans</i> zone	<i>S. radicans</i> , <i>Obione</i> , and <i>S. herbacea</i>	0.95
<i>Salicornia annua</i> zone	<i>S. annua</i> , <i>Pelvetia</i>	1.35
" " "	" " "	1.88
" " "	" " "	1.908
" " "	" " "	0.95
" " "	" " "	6.342
" " "	" " "	3.060
" " "	" " "	1.19
Aster Society, edge	Bare patch	0.97
" " centre	<i>A. tripolium</i>	1.33

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DESCRIPTION OF PLATE XV.

The headland as seen from the air. The various dune systems can be recognized by reference to Text-fig. 1. Note in particular the gradual coalescence and greater continuity of the plant covering as one passes from the youngest to the oldest dune systems.



For description see p. 431 and Text-fig 1, p. 393

On the Preparation and Use of Collodion Osmometers.

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IN an earlier paper¹ the writer described methods of preparing and grading collodion membranes for purposes of dialysis. It was there shown that by soaking the air-dried membranes in alcohol-water mixtures of different strengths and subsequently washing in water a series of membranes with a wide range of permeability could be obtained. At the one end of the series (using a grading mixture with a low concentration of alcohol) were membranes which were only slowly permeable to water and in a less degree to the simpler electrolytes, such as sodium chloride, potassium nitrate, &c.; at the other end (using a grading mixture with a high alcohol content) were those which allowed a slow diffusion of starch, aniline blue, and other substances which are highly colloidal in aqueous solution. Between these extremes any desired degree of permeability could be obtained. As was pointed out in the paper already referred to collodion membranes of certain grades are directly applicable to osmotic work, as it is possible with certainty to prepare them of such a permeability that they will allow a ready passage of water and at the same time hold back such solutes as cane sugar, copper sulphate, &c. They may thus be used to replace the well-known precipitation membranes (such as the copper ferrocyanide membrane of Traube and Pfeffer), which repeated attempts in this laboratory have shown to be difficult to prepare satisfactorily. The type of osmometer described in this paper has been used now for some years in the plant physiology practical classes in this laboratory, and has been found to be accurate and simple in manipulation. The following practical notes will serve to show how these osmometers are prepared and used. For convenience in description we shall deal in succession with:

1. Preparation of air-dried membranes or 'thimbles'.
2. Grading and method of attachment.
3. Method of using.

1. The type of membrane most convenient for the present purpose is that known as the thimble, which may be formed either inside or outside

¹ Biochem. Journ., 1915, ix, p. 591.

a test-tube. A full account of the method of preparation has been given in the paper already cited, and it is unnecessary to repeat the details here. Though the writer prefers to make the thimbles on the outside of the test-tube, as greater uniformity in successive membranes can be obtained by that method, it is probable that the alternative method will be found preferable for general purposes, as a certain amount of practice is required for success in stripping the membranes from the outside of the tube. A brief account was given in the original paper of a method of preparing membranes inside the test-tube, and it was stated then that these membranes tended to be too thin and fragile for practical purposes. Subsequent work has, however, shown that that difficulty can be overcome.

Schering's celloidin was formerly used in this work; latterly a preparation known as 'Necoloidine', and supplied by the New Explosives Company, has been found to be equally satisfactory. For preparing membranes by the 'inside' method, the arrangements given on p. 615 of the paper already mentioned will be found to give good results with the following alterations. Use a stronger solution of collodion (12 per cent.), and reduce the time of drainage to about two to three minutes. The membranes tend to be somewhat thin at the upper (closed) end, but this tendency can be checked by careful warming of the upper end of the tube during drainage. This has the effect of vaporizing some of the ether from the upper part of the membrane and preventing further running of the collodion sol in that part.

After drainage the membrane is dried off completely by blowing air into the tube. It is then separated from the tube by filling the latter with water when it is found that the collodion membrane readily separates from the glass. The membrane is now dried off completely, when it is ready for the next stage.

The important points to observe in drying the membrane inside the tube are: (1) not to touch the membrane until drying is well advanced, otherwise a hole will result; (2) to continue blowing in air until the membrane is well dried and may in fact begin to separate from the tube of its own accord. If the water is added before drying is well advanced, the subsequent drying in the open air will entail considerable shrinkage and distortion.

2. The membrane in its air-dried form is highly impermeable, and therefore, from the point of view of an osmometer, insensitive. To increase its permeability without at the same time making it permeable to the more slowly diffusible crystalloids, it is placed for twenty-four hours in (about) 60 per cent. alcohol—the maximum percentage varies somewhat with different makes of celloidin—then washed in several changes of water until the alcohol is removed. It must be emphasized that from the time the membrane is taken out of the grading mixture of alcohol and water it must not be allowed to undergo any drying. If dried out, it will of course revert to its previous highly impermeable condition.

In setting up this membrane as an osmometer use is made of the fact that whereas collodion will not adhere to glass in presence of water, it will under these circumstances adhere strongly to rubber. For the present purpose, rubber tubing is preferable to the ordinary rubber cork as the cylindrical shape of the former permits of a better joint. The method of procedure is given below.

A piece of pressure rubber tubing, about 1 inch long, is inserted into the open end of the thimble so that the edge of the membrane is about half-way up the 'cork'. The rubber tubing is chosen of such a diameter that it can just be inserted. A piece of ordinary glass tubing (about 2 to 3 inches long) is now passed through the cork until its inner end is flush with the inner surface of the latter. If the various parts have been suitably selected, the cork will be closely pressed against the membrane, all irregularities of which are thereby evened out so that a tight junction is effected. The membrane, filled with water to just below the lower end of the cork, is now set upright in water, so that the water-levels inside and outside are approximately the same. In this way the part of the membrane in contact with the rubber cork is allowed to dry out completely by exposure to the air, after which the junction is painted over with a collodion solution. This is in turn allowed to dry off and the junction is now watertight. A short length of capillary tubing is attached, and the osmometer is complete.

The simplest method of filling the osmometer is by pinching the membrane to drive out the air and running in the required solution from a funnel. The operation of filling must be carried out rapidly to avoid any risk of drying the membrane. For accurate work several fillings and emptyings are required each time a new solution is tested in the osmometer. As the membrane substance is tough it will stand repeated fillings and emptyings without damage, granted moderate care is exercised. Before using an osmometer for the first time it is advisable to keep it for a few days under an internal pressure of about two feet of water in order to even out any wrinkles that may be present, and to make sure that the membrane is properly sealed to the cork.

3. These osmometers are used in class work in the following manner. They are filled with the solution the osmotic pressure of which is to be determined (either unknown concentrations of cane sugar or copper sulphate, or a plant extract such as beet juice), the level of the meniscus being arranged somewhere in the middle of the capillary tube. The osmometer is now placed in a succession of dilutions of a standard cane-sugar solution, and by a process of trial and error one determines at what degree of dilution of the standard solution the meniscus remains steady. For class purposes the normality of the unknown solution is determined to within one unit in the second decimal place but a greater degree of accuracy than this is feasible. The method of procedure described below is more systematic, and

is recommended on account of the ease and rapidity with which the osmotic pressure of the unknown solution can be determined.

The determinations now to be described were carried out with a moderately thin osmometer of 7 c.c. capacity, attached to a capillary tube of 1 mm. internal diameter. For testing purposes a half-molar solution of copper sulphate was prepared, and the accuracy of the method determined by comparing known dilutions of this stock solution with each other.

In determining the osmotic strength of an unknown solution it is of great assistance to know certain constants appertaining to the particular osmometer. These should be determined when the osmometer is first used, for it will be found that a knowledge of them will greatly facilitate all future work with that particular osmometer.

The rate of passage of water through the membrane is proportional to the difference of osmotic pressure of the liquids on opposite sides. If, therefore, the rate of passage corresponding to a known difference has been determined, it is possible to tell the whereabouts of an unknown concentration by a single test of it against a known solution. The concentration suggested by this first reading can now be set up and compared with the unknown solution. By this means a closer approximation is obtained at the second trial, and thus one rapidly arrives at the concentration of the unknown solution.

With the particular osmometer under consideration, and with half-molar copper sulphate inside and water outside, the rise of the meniscus in 1 hour is 3.3 cm. at 17° C.

The rate of intake of water varies considerably with temperature, as is shown by the following figures :

<i>Temperature.</i>	<i>Rise of meniscus in 1 hour.</i>
14°	3.0
17°	3.3
24°	4.4

Thus a constant temperature bath, if available, is advantageous, but if laboratory temperature does not vary more than a few degrees, and if a rough correction is made for this temperature effect, the rapidity with which one obtains a close approximation to the unknown solution is not seriously affected.

In addition to the effect of temperature on the rate of endosmosis, account is to be taken of its more direct effect on the osmometer. In the present case the meniscus was found to rise 1.5 mm. for each 1° C.

In dealing with a solution of an electrolyte such as copper sulphate it is well to remember that the osmotic pressure is not directly proportional to the concentration of salt present. As it is a function of the number of molecules plus ions present, and as the degree of ionization depends on concentration (in accordance with the law of mass action), one can easily prove

that the osmotic pressure of an electrolyte is not a linear function of the concentration, but falls short of a linear relation as the concentration rises. Thus the osmotic pressure of half-molar copper sulphate is less than twice that of a quarter-molar solution. The following determinations with this particular osmometer illustrate this point :

<i>Concentration inside.</i>	<i>Concentration outside.</i>	<i>Difference</i>	<i>Endosmosis.</i>	<i>Endosmosis ÷ concentration difference.</i>
$n/2$	0	$n/2$	3.3	3.3
$n/4$	0	$n/4$	1.8	3.6
$n/2$	$n/4$	$n/4$	1.5	3.0

Thus a given increase of concentration produces a greater effect on the osmotic pressure when the concentration is low than when it is high. Attention to this fact enables one to approach more rapidly the concentration of an unknown solution.

A particular determination will now be described.

A solution, x , of concentration unknown to the experimenter, was prepared by dilution of the stock half-molar copper sulphate solution. The following readings were then obtained :¹

<i>Inside.</i>	<i>Outside.</i>	<i>Rise in 1 hour at 17°.</i>
$m/2$	water	3.3 cm.
$m/2$	x	0.65, 0.7

Assuming as a first approximation that osmotic pressure is directly proportional to concentration, we find that the value of x is in the neighbourhood of 0.40 molar ($\frac{m}{2} - \frac{0.7}{3.3} \times \frac{m}{2}$). In view of the effect of ionization above mentioned, this value is too high. A slightly weaker solution than 0.40 molar was therefore set up, i.e. a 0.38 molar solution. The solution x was now put inside the membrane and the 0.38 solution outside, with the following result :

Temperature at start = 17.0°.

After 18 hours, rise = 0.3 cm. Temp. = 14.0°.

The rise in 18 hours (corrected for temperature) was therefore approximately 0.75 cm. Calculating as above, we find the approximation to the unknown solution to be 0.385 molar. This concentration was now made up and tested against the x solution. A slight drop, amounting to 0.2 cm. in 24 hours, was recorded. Using this correction as before, the final value obtained was 0.384 molar. The correct value according to the original measurements was 0.383 molar. Thus with three trials the molecular strength of the unknown solution was found correct to one unit in the third decimal place. In a second experiment, a solution was estimated as 0.368 molar as against the correct figure of 0.366 molar.

¹ In any determination the whole of the membrane must be completely submerged in the outside solution.

It may be said generally that by means of three or four determinations performed as above the method will give the correct molecular value to within two units in the third decimal place. This degree of accuracy is thus somewhat greater than that claimed by Barger¹ for his method—viz. five units in the third decimal place. Each method has its own particular merits, but the present method has the great advantage for demonstration purposes that it measures osmotic pressure directly, i.e. by diffusion across a semipermeable membrane, and not indirectly through the effect of osmotic pressure of the solute on the vapour tension of the solvent. It thus illustrates more clearly the functioning of the living cell as an osmotic unit.

The carrying out of the above determinations requires about two days. In the course of the finer determinations which run over a period of about twenty-four hours the solutions must be protected against evaporation. It is advisable to have the unknown solution inside the osmometer during the longer periods, as it is thereby protected from evaporation, and in any case its concentration can be brought back to its original value. It is probable that an accuracy of 0.002 molar is about the best that can be obtained short of elaborate arrangements for preventing change in concentration through evaporation. It also approaches very close to the limits of accuracy which usually obtain in solutions prepared for quantitative work.

In taking the above readings, the level of the meniscus was kept at about 10 cm. above the outside level. This head of water represents a molecular concentration of about 0.0005, and thus if greater accuracy is attempted than that claimed above, this factor will require to be allowed for.

In order to bring back the meniscus in the capillary to the same point, and thus ensure that no alteration in concentration has taken place either by evaporation or by diffusion of water across the membrane, the osmometer is placed in water if the level has fallen, or, in the case where the level has risen, first washed on the outside with water, the excess of water drained off by means of filter-paper and the contents of the osmometer allowed to concentrate to the required degree by evaporation of water through the membrane. This latter process can be repeated time and again without affecting the permeability of the membrane; that is, no drying out of the membrane takes place at ordinary temperatures so long as one side of it is maintained wet.

Strictly speaking, these osmometers can only be used to determine the osmotic pressure of solutions the solutes of which do not diffuse across the membrane. As, however, the diffusibility of water is much greater than that of even the simplest electrolytes, considerable accuracy can be obtained even in the case of such substances.

It will be noticed that the method of determining osmotic pressure here described is one of comparison and not of absolute measurement. For

¹ Journ. Chem. Soc. (Trans.), 1904, lxxxv, 286-324.

most biological purposes the former is sufficient, as the osmotic pressure of a biological fluid may be determined in terms of a standard substance such as cane sugar, and thereby obtained either from tables or from theoretical calculation. For the absolute measurement of osmotic pressure (except where it is very small, as in the case of colloidal solutions) these osmometers cannot be used, as they stretch under moderate pressure. Semi-permeable collodion membranes in sheet form could, however, be so used, but some type of apparatus to take up the pressure developed would obviously be necessary.

These osmometers, as far as is known, last indefinitely. If kept in water they tend to become coated with fungoid growths so that they should be kept in a dilute solution of an antiseptic, e. g. dilute copper sulphate.

The Gametophyte and Embryo of *Botrychium simplex*, Hitchcock.

BY

DOUGLAS H. CAMPBELL.

With Plate XVI and ten Figures in the Text.

BOTRYCHUM SIMPLEX, Hitchcock, is the smallest species of the genus, and is reported from various stations in northern Europe, Japan, and North America,¹ being decidedly a boreal species. In some of its forms it shows a striking superficial resemblance to a small *Ophioglossum*, while the larger forms are much like *B. lunaria*, to which it is undoubtedly not very remotely related. There seems to be no doubt, however, that it is a valid species.

Luerssen² figures a very interesting series of forms, but thinks that these represent, to a great extent, only different ages. The second leaf of the young sporophyte, although very small, nevertheless is fertile, and it is probably several years before the sporophylls reach their full development. Text-fig. 1 shows two extremes found by the writer in a number of specimens collected by Dr. H. C. Lyon, to whom the writer is indebted not only for these specimens, but also for the material of the gametophyte and young sporophytes upon which the present paper is based.

Dr. Lyon has sent the writer the following account of the conditions under which the material was collected in Minnesota:

My material of *Botrychium obliquum* and of *B. simplex* was collected in swampy ground on the shores of Echo Lake. This is a small lake a few miles from White Bear Lake. The soil in this district is mostly sand and carries a sparse forest of white oak, bur oak, red oak, black oak, birch and poplar. All three species of *Osmunda* occurred here in great abundance. *Lycopodium obscurum* and *L. inundatum* occur here, while the only known station of *Ophioglossum vulgatum* in this state is at this point. I collected *Botrychium matricariaefolium* gametophytes only a few rods from the point where those of *B. simplex* were found. *Isoetes* occurs abundantly in the shallow water of Echo Lake. You will note from the above that this region

¹ Christensen, C.: Index Filicum, 1906.

² Luerssen, C.: Die Farnpflanzen oder Gefässkryptogamen, Fig. 181, Leipzig, 1889.

carries a remarkable Pteridophytic flora. It is also remarkable in that many of the flowering plants of the northern woods occur here. The orchid flora is particularly rich and interesting.'

The gametophyte of *B. simplex* is much like that of the other species that have been described,¹ being on the whole most like that of *B. lunaria*,

as might be expected. Bruchmann describes the latter as a small subterranean tuber-like body, 1–2 millimetres in length. In all these respects *B. simplex* agrees closely with *B. lunaria*, except that some of the specimens examined by the writer were slightly larger than the dimensions given for *B. lunaria*, reaching a maximum length of about three millimetres. Bruchmann shows figures of several cases where there apparently had been a dichotomy of the apex of the gametophyte, which had two short divergent branches, so that the gametophyte was heart-shaped. This condition was not seen in *B. simplex*, but it is quite possible that it may occur.

In both *B. simplex* and *B. lunaria*, which belong to the section *Eubotrychium*, the gametophyte is much smaller than in either *B. obliquum* or *B. virginianum*, representing respectively the sections *Sceptridium* and *Osmundopteris*.²

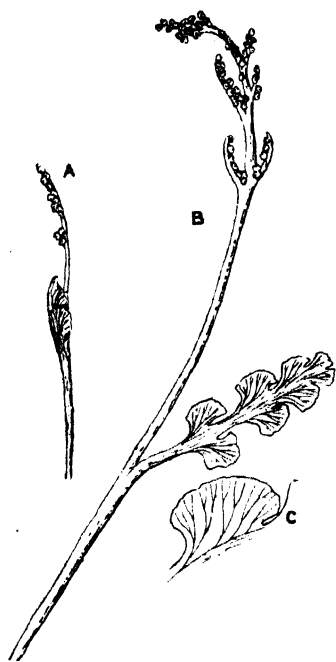
Fig. 1 of Plate XVI shows one of the youngest specimens found in the material examined. Seen from above it is nearly oval in outline, slightly pointed at the apex, which

is nearly colourless, while the older portions are brownish in colour. Short dark-brown rhizoids are present, but these are neither so abundant nor so long as Bruchmann figures for *B. lunaria*. These rhizoids are still less conspicuous in older stages, such as that shown in Fig. 2.

As in the other species of *Botrychium* the gametophyte is decidedly dorsiventral in structure, somewhat flattened, and with a more or less conspicuous median ridge upon which the antheridia are borne. The latter begin to develop when the gametophyte is very small, and new ones arise in

¹ Bruchmann : Über das Prothallium und die Sporenpflanze von *Botrychium lunaria*, L. Flora, 1906; Jeffrey, E. C.: The Gametophyte of *Botrychium virginianum*. Proc. Canad. Inst., 1898, 5; Campbell: The Eusporangiateae. Carnegie Institution of Washington, Publication No. 140, 1911.

² Campbell, Gametophyte and Embryo of *Botrychium obliquum*, Mühl. Ann. of Bot., xxxv. 157, 1921.



TEXT-FIG. 1. A, B. Two specimens of *Botrychium simplex*, Hitchcock, collected by Dr. H. L. Lyon at Echo Lake, Minnesota. Natural size. C. Leaf segment showing venation. $\times 3$.

acropetal succession for a long time. While the archegonia are somewhat later in making their appearance, still the first ones are formed while the gametophyte is still very young. They do not develop upon the median ridge, but form a line on either side of it (Plate XVI, Figs. 1, 2, 5, 6).

Sections of the gametophyte (Figs. 3, 5) show it to be composed of nearly uniform parenchyma, which in the older portions of the thallus is infested with the characteristic endophytic fungus found in all the *Ophioglossaceae*. In *B. simplex* the endophyte seems to be somewhat less uniformly distributed than is usually the case, and a good many cells of the infected area are nearly or quite destitute of the fungus, while in others the hyphae form dense tangled masses or clumps within the cell. No special study was made of the endophyte, as there was no indication that it differs materially from that which has been described in other species.¹

As usual, the fungus is confined to the older tissues of the gametophyte and does not invade the meristematic tissues, nor the immediate vicinity of the reproductive organs.

The apex of the gametophyte shows a well-marked growing-point (Fig. 3, α). This is slightly inclined towards the dorsal surface of the thallus, the ventral tissue extending somewhat beyond it. In several cases there was evident in median sections a cell (Fig. 4, α), which, from its form and position, closely resembles the apical cell of many liverworts, and probably is the definite simple apical cell of the thallus. No successful horizontal sections of the growing-point could be made, so that the exact form of this apical cell was not determined.

ANTHERIDIUM.

As will be seen from the figures, the first antheridia appear very early, and others arise in acropetal succession for a long period. They agree closely with those of the other species in their development.

The first division in the mother-cell separates an outer, or cover-cell, from an inner one which gives rise to the mass of spermatocytes (Figs. 7, 8). The cover-cell undergoes a series of divisions by vertical walls, and most of the resultant cells undergo a transverse division, so that the outer wall of the antheridium is composed, for the most part, of two layers of cells; but in one of the cells the transverse wall is suppressed, and this cell (ϕ . in Fig. 9) becomes the operculum, which is destroyed when the antheridium opens and allows the spermatozoids to escape.

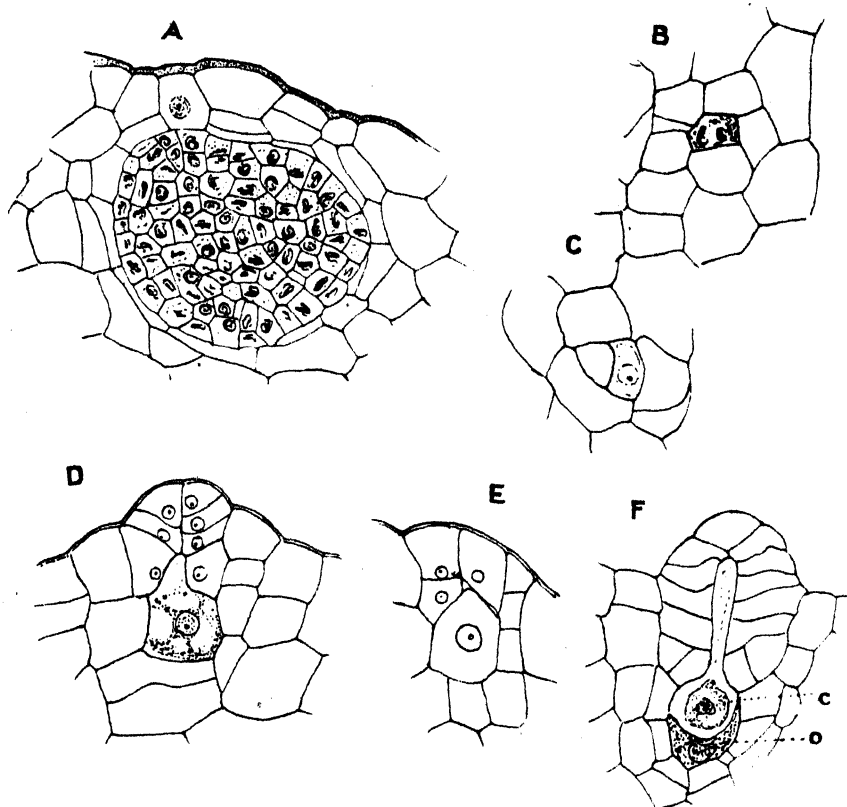
The operculum, seen from the surface (Text-fig. 2, B), is nearly square, but occasionally there is a suggestion of the triangular operculum found in *Ophioglossum*,² a condition also occasionally found in *B. obliquum*. In the latter species, two opercular cells are not uncommon, and Bruchmann shows

¹ See Jeffrey, loc. cit.; Campbell, The Eusporangiatae.

² Campbell, The Eusporangiatae, Figs. 12, 13.

three in an antheridium of *B. lunaria*. It is very likely that more than one may also occur in *B. simplex*, but this was not the case in any of the specimens examined.

The divisions in the inner cell of the young antheridium follow the usual sequence. The first two divisions are at right angles, forming four equal cells, which later divide more or less irregularly until a large number



TEXT-FIG. 2. A. Median section of nearly ripe antheridium. $\times 300$. B, C. Surface view of two antheridia showing the operculum. $\times 300$. D. Median section of archegonium before the separation of the central and neck canal-cells. $\times 300$. E. Young archegonium showing irregular division in the cap-cell. F. Archegonium in which the egg-cell, *e*, is much compressed, and the lower part of the canal-cell, *c*, is enlarged so as to resemble an egg-cell.

of polyhedral cells result—the spermatocytes. So far as could be determined from a somewhat cursory study, the spermatogenesis does not differ from that of *B. virginianum* and *Ophioglossum*.¹ We may safely assume that the greater part of the spermatozoid is derived from the nucleus of the spermatocyte, while the anterior cilia-bearing portion arises from the blepharoplast.

Of course it was not possible to examine living spermatozooids; but

¹ Campbell, *The Eusporangiateae*.

not infrequently, in sections, recently opened antheridia were encountered within which were found free spermatozooids. These showed quite satisfactorily the form of the complete spermatozoid (Fig. 10). It is a rather thick, somewhat flattened band, consisting of about two coils, the larger posterior one containing most of the nuclear substance; the smaller anterior coil is made up mainly of the blepharoplast, to which are attached the cilia. Except for their somewhat smaller size they closely resemble those of *B. obliquum*. The antheridia are also somewhat smaller than those of the latter species, but, to judge from Bruchmann's figures, are larger than those of *B. lunaria*, and this is true also of the spermatozooids.

THE ARCHEGONIUM.

The archegonium of *B. simplex* differs but little from that of the other species. As already mentioned, they are situated on the dorsal surface of the thallus, on either side of the antheridial ridge, but are not developed upon the ridge itself (Figs. 1, 2). In their earliest stages (Fig. 11) they are hardly distinguishable from young antheridia, but very soon their real nature becomes apparent. As in all the Ophioglossaceae, a basal cell is usually absent. The central cell becomes convex above and grows out into a slender prominence which extends between the elongating neck-cells (Fig. 12; Text-fig. 2, D), and later is cut off to form the elongated neck canal-cell (Figs. 13, 14). The separation of the central cell and neck canal-cell takes place somewhat earlier in *B. simplex* than it does in *B. obliquum*.

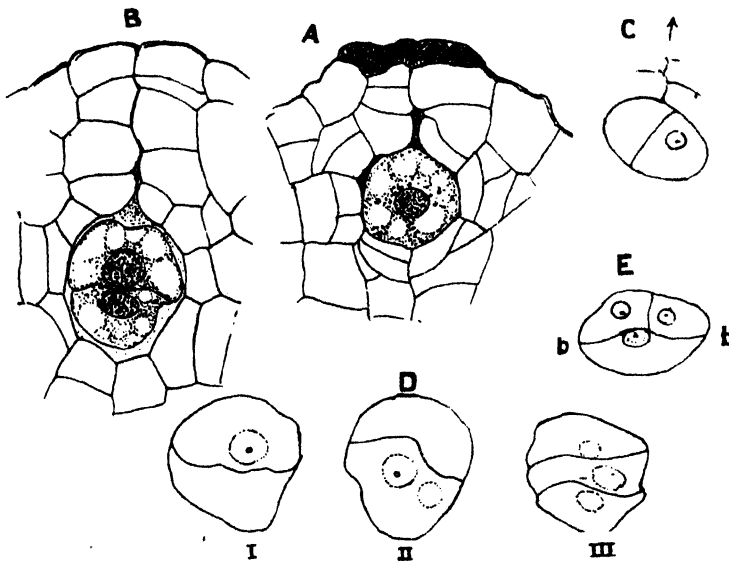
The nucleus of the canal-cell divides into two, one remaining in the broad basal portion, the other being near the apex (Fig. 14). In no cases was there any division wall seen between the two nuclei.

As in all the other Ophioglossaceae, the presence of a ventral canal-cell, such as usually is found in the ferns, is difficult to demonstrate. In several instances there was present in the central cell of the mature archegonium a small body, apparently a second nucleus (Fig. 15, v). This nucleus, if such it was, did not stain as strongly as the egg nucleus, nor was there any conclusive evidence that it was the result of a division of the nucleus of the central cell. A similar condition was seen by the writer in other Ophioglossaceae, but it must, for the present, remain an open question whether this nucleus (?) represents the ventral canal-cell usually found in the fern archegonium.

Each of the four primary neck-cells divides, by successive transverse walls, into about six to eight cells, of which about half extend above the surface of the thallus (Fig. 14). Sometimes, apparently the result of the obliquity of one of the first divisions in the cover-cell of the young archegonium, there is a certain lack of symmetry evident (see Fig. 13; Text-fig. 2, e).

THE EMBRYO.

The genus *Botrychium* shows a remarkable diversity in the structure of the embryo and young sporophyte, and the writer has recently had occasion to call attention to this. While in *B. obliquum* and *B. virginianum* the cotyledon is remarkably well developed, in *B. lunaria* the cotyledon is reduced to an insignificant rudiment, and several more subterranean leaf rudiments are formed before the first foliage leaf appears above ground. *B. obliquum* differs from the other known species in the presence of a conspicuous suspensor, discovered by Lyon.¹ The embryo of *B. simplex*, as might be expected, more nearly resembles that of *B. lunaria*, to which



TEXT-FIG. 3. A. Fertilized archegonium containing a unicellular embryo. $\times 300$. B. Two-celled embryo. $\times 300$. C. Two-celled embryo, showing oblique basal wall. $\times 300$. D. Three longitudinal sections of a four-celled embryo showing no vertical divisions. $\times 300$. E. Longitudinal section of three-celled embryo. $\times 300$.

it is undoubtedly pretty closely related, than it does either *B. obliquum* or *B. virginianum*. However, there are differences, especially the much better developed cotyledon, which distinguish it from *B. lunaria*. How constant these differences are must remain for the present somewhat uncertain.

As in *B. lunaria*, the embryo may begin to develop while the gametophyte is extremely small, and often several archegonia are fertilized at about the same time, and begin to develop the embryos. As a rule at least, only one embryo develops beyond the earliest stages, and reaches maturity. Unicellular and two-celled embryos were frequently encountered, but the later stages were very much less common.

¹ Lyon, H. C.: A New Genus of Ophioglossaceae. Bot. Gazette, xl, 1905.

Bruchmann figures several cases in *B. lunaria* where two young sporophytes were developed from the same gametophyte; but no such cases were seen in *B. simplex*, although of course it is quite likely that this might sometimes occur.

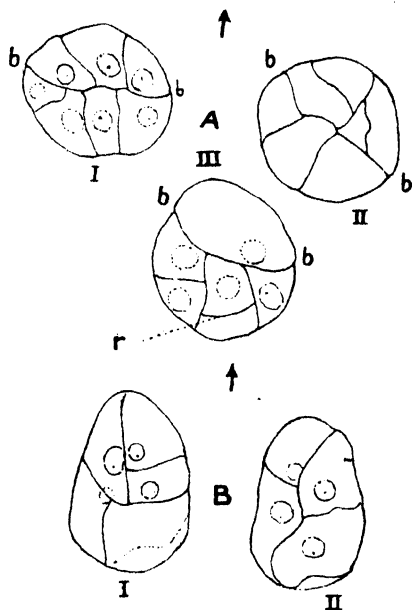
After fertilization, the free portion of the archegonium neck usually breaks down. The fertilized egg develops a membrane and the unicellular embryo soon completely fills the cavity of the venter (Text-fig. 3, A). It soon divides by a transverse wall, the two cells usually being of approximately equal size, but sometimes one is longer than the other (Text-fig. 3, B).

To judge from a not very complete series of embryos secured by the writer, *B. simplex* shows an unusual amount of variation in the early division of the embryo. While the first (basal) wall is generally transverse, as it is in most Eusporangiateae, it may be strongly oblique (Text-fig. 3, C), and the succeeding divisions are extremely variable.

In form, the young embryo may be globular or somewhat elongated either vertically or horizontally (see Text-fig. 3). The succeeding divisions evidently are extremely variable, but the number of young embryos available was too small to make it possible to determine what is the most common arrangement of the cells in the very young embryo. Bruchmann shows a regular quadrant and octant division for *B. lunaria*, but none of the embryos of *B. simplex* showed such regularity in the position of the walls of corresponding stages.

Text-figs. 3, D, E, show two of the youngest embryos seen by the writer. E is a three-celled stage, in which the epibasal portion is divided by a median (quadrant) wall, the hypobasal part being still undivided. D shows three longitudinal sections of a four-celled embryo, in which no vertical walls have yet been formed.

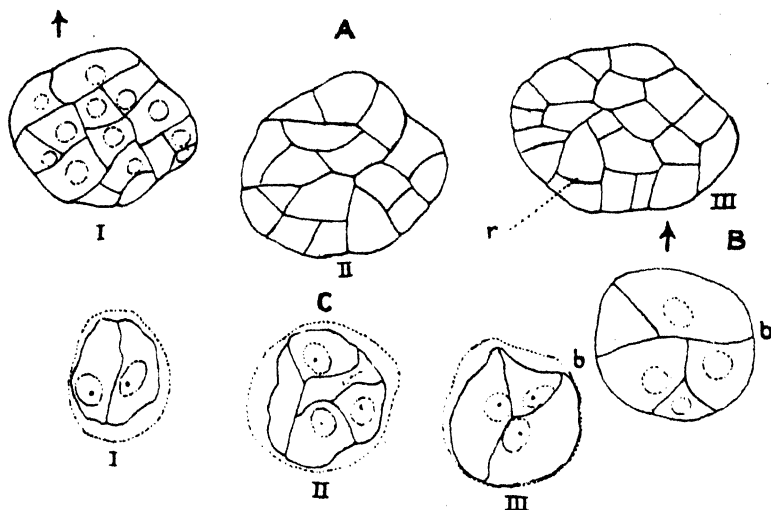
Text-fig. 4, A, shows three sections of an older stage in which there is an approach to a quadrant division, and this embryo corresponds pretty closely with that of *B. lunaria* shown in Bruchmann's Fig. 39. It is not possible to determine at this stage the relation which these early divisions



TEXT-FIG. 4. A. Three longitudinal divisions of a young embryo showing a pretty regular quadrant division; *b, b*, basal wall; *r*, root-initial (?). $\times 300$. B. Two somewhat oblique sections of an embryo of about ten cells. $\times 300$.

bear to the organs of the young sporophyte. It is possible that *r* in section III may be the beginning of the primary root, but this is by no means certain.

Text-fig. 4, B, shows two somewhat oblique longitudinal sections of an embryo with about ten cells. This embryo was decidedly elongated vertically instead of having the globular form of the embryo shown in A. It was somewhat pointed above, a condition which is sometimes found also in *B. virginianum*. Indeed, it may be said that in general the earliest stages of the embryo in *B. simplex* resemble more nearly the corresponding stages in *B. virginianum*¹ than those of *B. lunaria*.



TEXT-FIG. 5. A. Three longitudinal sections of an embryo in which the basal wall is no longer certainly visible. The cell *r*, in III, may be the root-initial. $\times 300$. B. Young embryo showing the basal wall, *b*, clearly. C. Three transverse sections of a young embryo; I, section next the archegonium. $\times 300$.

A young embryo of seven cells (Text-fig. 4, B) had two cells in the epibasal portion and five in the hypobasal, the latter quite irregularly disposed. The resemblance to *B. virginianum* is also shown by a comparison of the transverse sections. Text-fig. 5, C, shows such a series, and it is much like a similar series of *B. virginianum*, shown in Fig. 31 of the writer's monograph of the Eusporangiateae. In both cases the section next the archegonium was composed of two equal cells, showing an absence of octant walls in the epibasal region.

In a somewhat older stage (Text-fig. 5, A) there is a large cell, *r*, in the hypobasal region which in form and position suggests that it may be the initial of the primary root; but as no stages were found between this and the very much older embryo shown in Text-fig. 6, in which the apical cell of the root was plainly visible, this is by no means certain.

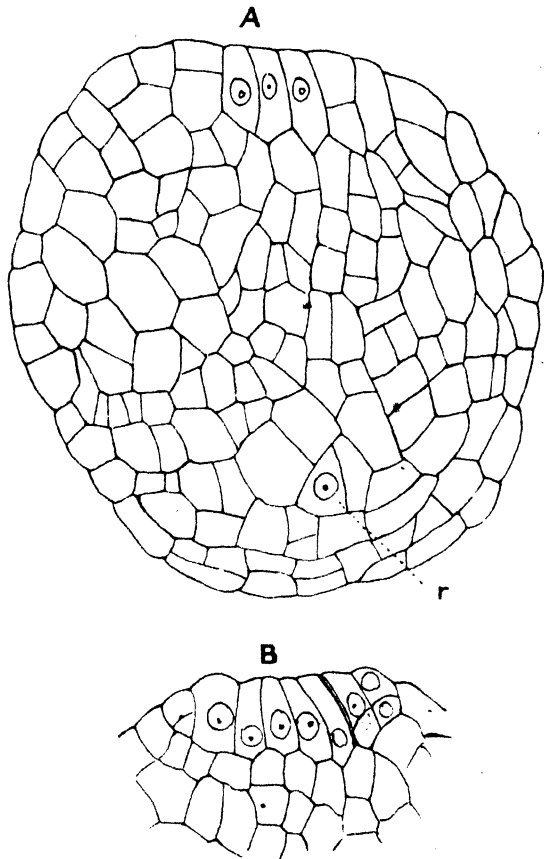
¹ See Campbell, *The Eusporangiateae*, Figs. 28-31.

Bruchmann's figures of such intermediate stages in *B. lunaria* do not show any evident root-initial, which is first shown in much older embryos. In corresponding stages of both *B. obliquum* and *B. virginianum* the writer has found the initial cell of the primary root to be readily demonstrable. In the former species, however, the initial cell is developed later than in *B. virginianum*, and originates near the centre of the embryo, very much as in the Marattiaceae and *Ophioglossum*. In *B. virginianum* the root-initial is cut off from a superficial cell, much as in the leptosporangiate ferns. It is, in fact, very much like the cell *r* shown in Text-fig. 5.

The large embryo shown in Text-fig. 6 appears to be somewhat younger than Bruchmann's Fig. 42, but it shows decidedly more differentiation of the parts than Bruchmann's figures of *B. lunaria* exhibit. In the latter, apparently the only sign of differentiation in the embryo at this time is the presence in the axis of what looks like the beginning of a strand of procambium. The apex of the root seems to be quite undifferentiated.

In *B. simplex*, at this stage, the apical cell of the root, *r*, is clearly evident, and the apex of the embryo is occupied by a group of columnar meristem cells, one of which is probably the initial for the stem-apex, and possibly one may represent the apex of the cotyledon; but it is not possible to be sure of this. There is a slight indication of the formation of an axial procambium strand, but, unlike *B. lunaria* and *B. virginianum*, the foot, as such, is not clearly delimited, and the embryo at this stage suggests the bipolar embryo of *B. obliquum*.

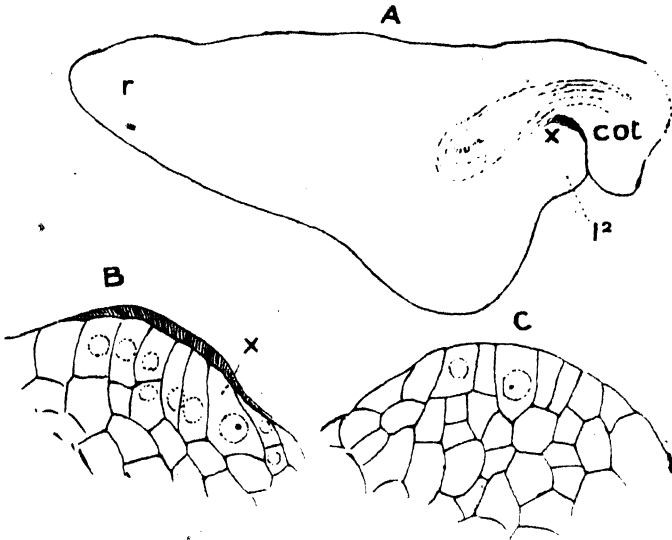
The writer was unable to secure any stages between this embryo and



TEXT-FIG. 6. A. Nearly median section of a large embryo showing the initial of the root, *r*, and the apical meristem. $\times 300$. B. The apical meristem from another section of the same embryo.

the very much older one shown in Text-fig. 7, in which the root had emerged from the gametophyte and the stem-apex and cotyledon were already well developed. It is, however, clear that the differentiation of the primary root and stem-apex occur much earlier in *B. simplex* than in *B. lunaria*, and in this respect *B. simplex* is more like *B. virginianum* or *B. obliquum* than it is like *B. lunaria*.

Bruchmann's Fig. 43 represents a stage intermediate between our Text-fig. 6 and the much older one shown in Text-fig. 7. This embryo of *B. lunaria* has the apical cell of the root clearly defined, and the stem-apex is recognizable, occupying a narrow cleft between the base of the extremely



TEXT-FIG. 7. A. Nearly median section of a young sporophyte, in which the root has just emerged; the root, *r*, is cut obliquely; *cot*, the cotyledon; *x*, apex of the stem; *l*², second leaf. $\times 45$. B. Apex of the stem; *x*, the apical cell. $\times 300$. C. Apex of second leaf. $\times 300$. Sections made by Dr. L. Baas-Becking.

rudimentary cotyledon and the base of the massive root. At this stage the embryo consists almost entirely of the root and foot.

B. simplex differs notably from *B. lunaria* in the earlier appearance of the stem-apex and cotyledon, and the much better development of the latter. The fully developed cotyledon was not seen by the writer, but Dr. L. Baas-Becking, who has been engaged in a study of the more advanced stages of the young sporophyte, has informed him that the cotyledon, though much less conspicuous than that of either *B. obliquum* or *B. virginianum*, develops a small lamina and is apparently functional.

Text-fig. 7 shows a young sporophyte slightly older than that represented by Bruchmann's Fig. 45. The general relation of the parts is much the same, but the foot is less prominent in *B. simplex*, and the cotyledon and

stem-apex relatively much more important. The cotyledon is very much like that of *B. obliquum* at a similar stage of development, showing a very large basal sheath which encloses the stem-apex and the young second leaf. The upper part of the leaf, however, is less developed than in *B. obliquum*. It strikingly resembles the early stages of the leaves of the older sporophyte shown by Bruchmann in his Fig. 57.

The stem-apex of the young sporophyte (Text-fig. 7, B) shows a large apical cell, which differs from that of *B. lunaria* in having a truncate base. It thus closely resembles that of *B. obliquum* or *Ophioglossum*.

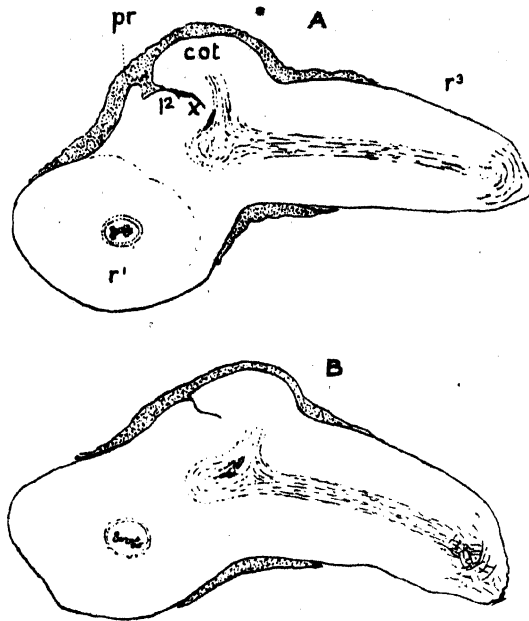
The second leaf (Text-fig. 7, C) forms a blunt cone, whose summit is occupied by a single large cell, presumably the apical cell of the more advanced leaf.

The primary vascular bundle is now clearly visible, extending from the cotyledon into the root. As the root makes a decided angle with the cotyledon, only the base of the root-bundle shows in the figure. Bruchmann shows exactly the same condition in *B. lunaria*, where, however, owing to the very rudimentary nature of the cotyledon, the bundle does not extend into it. While the development of the apical bud is decidedly more advanced in *B. simplex* than in *B. lunaria*, its further development, as in the latter, is slow when compared with the development of the root-system, and the general type of the young sporophyte is much more like that of *B. lunaria* than like that of *B. virginianum* or *B. obliquum*, where the cotyledon is so conspicuous.

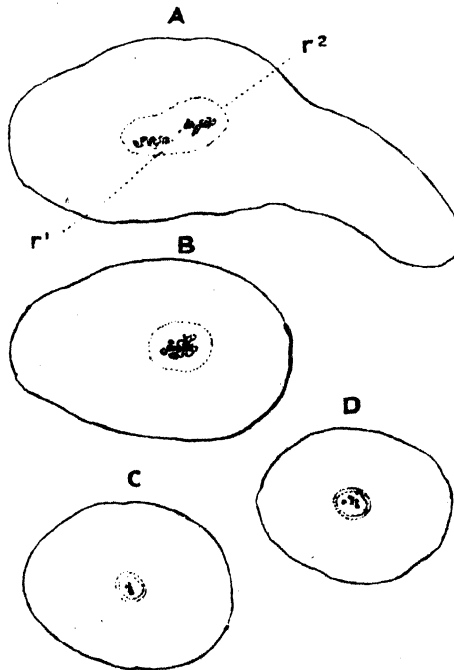
In *B. simplex*, while the cotyledon is still small, a second root develops, and soon after a third, while the apical bud remains quite inconspicuous (Fig. 16). This young sporophyte showed two large roots and a third smaller one, while the terminal bud, although quite small, was still more conspicuous than in similar stages of *B. lunaria*. The gametophyte (*pr.*) can still be seen. As will be seen from a section of this plant (Text-fig. 8, A), the cotyledon and stem-apex are scarcely more developed than at the time of the emergence of the primary root, but nevertheless are decidedly more so than in corresponding stages of *B. lunaria*.

The steles of the three roots unite near the centre of the sporophyte, where they are joined by the bundle from the cotyledon; but there is no stelar tissue belonging to the stem-apex. Near the junction of the cotyledonary bundle and that of the third root can be seen a group of tracheides which can be seen to connect also with the base of the second root. By examining a series of sections, this tracheary tissue can be found to merge with the xylem of the primary root, and forms a solid strand of xylem in the centre of the axis of the young sporophyte.

Near the base of the primary root (Text-fig. 9, D) two very unequal xylems appear, but nearer the apex of the root the smaller one disappears and the bundle becomes monarch.



TEXT-FIG. 8. Two sections of the sporophyte shown in Plate XVI, Fig. 16. *pr*, gametophyte; r^1 , r^2 , r^3 , the three roots; *cot*, cotyledon; r^2 , second leaf; *x*, stem-apex. $\times 40$.

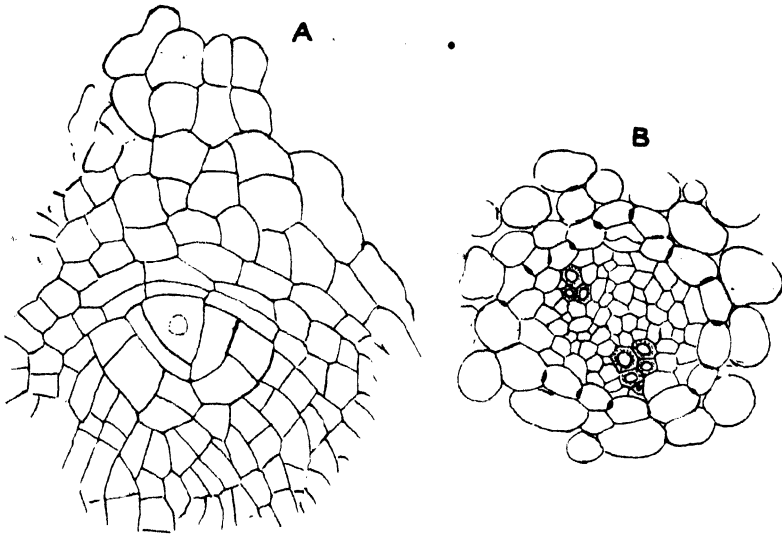


TEXT-FIG. 9. A, Another section of the same sporophyte as in Text-fig. 8, showing the approximation of the xylems from the first and second roots. $\times 40$. B, Another section showing the complete fusion of the xylems. C, Section of the first root showing single xylem. D, The same near the base, showing two very unequal xylems.

The second root of this plant (Text-fig. 10, B) was diarch, but the two groups of tracheides were unequal in size.

The apex of the third root (Text-fig. 10, A) shows the characteristic tetrahedral initial cell, with somewhat irregular segmentation. The root-cap is derived in part from direct segments cut off from the apical cell, and in part from periclinal divisions in the lateral segments.

The cotyledon probably emerges after about four roots have developed, and soon after, perhaps the next year, the second leaf appears above ground as a very small fertile frond (Figs. 17, 18, 19). The base of the petiole forms an elongated sheath, within which can be plainly seen the next



TEXT-FIG. 10. A. Median section of the apex of the third root. $\times 300$. B. Cross-section of the bundle of the second root. $\times 300$.

younger leaf. The lamina of the leaf is very small, with a slightly toothed margin and dichotomous venation. The sporangiophore is incurved and bears a very few sporangia. The one shown in Fig. 18 had only one perfect sporangium, and it may be that sometimes no perfect sporangia develop on this rudimentary sporophyll.

The relation of the sterile lamina and the sporangiophore was not investigated, but it is highly probable that the two arise by an early dichotomy of the leaf-apex, such as Bruchmann found to be the case in the later leaves in *B. lunaria*.

While the embryo of *B. simplex* agrees in the main with that of *B. lunaria*, the subordination of the foliar structures to the roots of the young sporophyte is much less marked. In *B. lunaria* Bruchmann states that at least seven, and sometimes as many as nine, rudimentary leaves are

formed, which never appear above ground, before the first functional leaf—a sporophyll—is developed.¹ He believes that only one leaf—even the rudimentary ones—is formed each year, but this seems rather unlikely.

As in *B. simplex*, it appears that in *B. lunaria* there is no cauline stele, but all the stelar tissues belong to the roots, or to the rudimentary traces of the early leaves.

SUMMARY AND CONCLUSION.

1. *Botrychium simplex*, the smallest species of the genus, is undoubtedly nearly related to *B. lunaria*, but is specifically distinct.

2. The gametophyte of *B. simplex* resembles closely in size and structure that of *B. lunaria*. It is much smaller than that of the other species of *Botrychium* that have been examined. There is probably a single apical cell present. The rhizoids are less developed than in *B. lunaria* and the endophytic fungus is less conspicuous.

3. The sexual organs are much like those of *B. lunaria*, but the antheridia and spermatozoids are somewhat larger.

4. The presence of a ventral canal-cell (or a nucleus representing this) is doubtful. This is the case also in other species of *Botrychium*.

5. More than one archegonium may be fertilized, but no cases were seen where more than one sporophyte was developed from the same gametophyte.

6. The embryo, on the whole, resembles that of *B. lunaria*; but the early divisions are variable and much more irregular, in which respect it is more like that of *B. virginianum*.

7. The apical bud of the young sporophyte is much better developed than in *B. lunaria*. The apical cell of the stem is not tetrahedral, but has a truncate base, as in *B. obliquum*. The cotyledon is functional, but much smaller than that of *B. obliquum* or *B. virginianum*. The second leaf is a sporophyll.

8. The root-system develops much more rapidly than the apical bud, but does not preponderate so much as in *B. lunaria*. The roots are of the ordinary type, with a tetrahedral apical cell. The first root may be monarch; the succeeding roots are diarch.

9. The vascular skeleton of the young sporophyte is made up exclusively of the steles belonging to the roots and leaves.

In spite of the much less regular divisions in the young embryo, *B. simplex* agrees pretty closely with *B. lunaria*. These members of the section *Eubotrychium* have a much smaller gametophyte than the other species that have been studied, and the young sporophyte is characterized by the preponderant development of the root-system compared with the foliar structures.

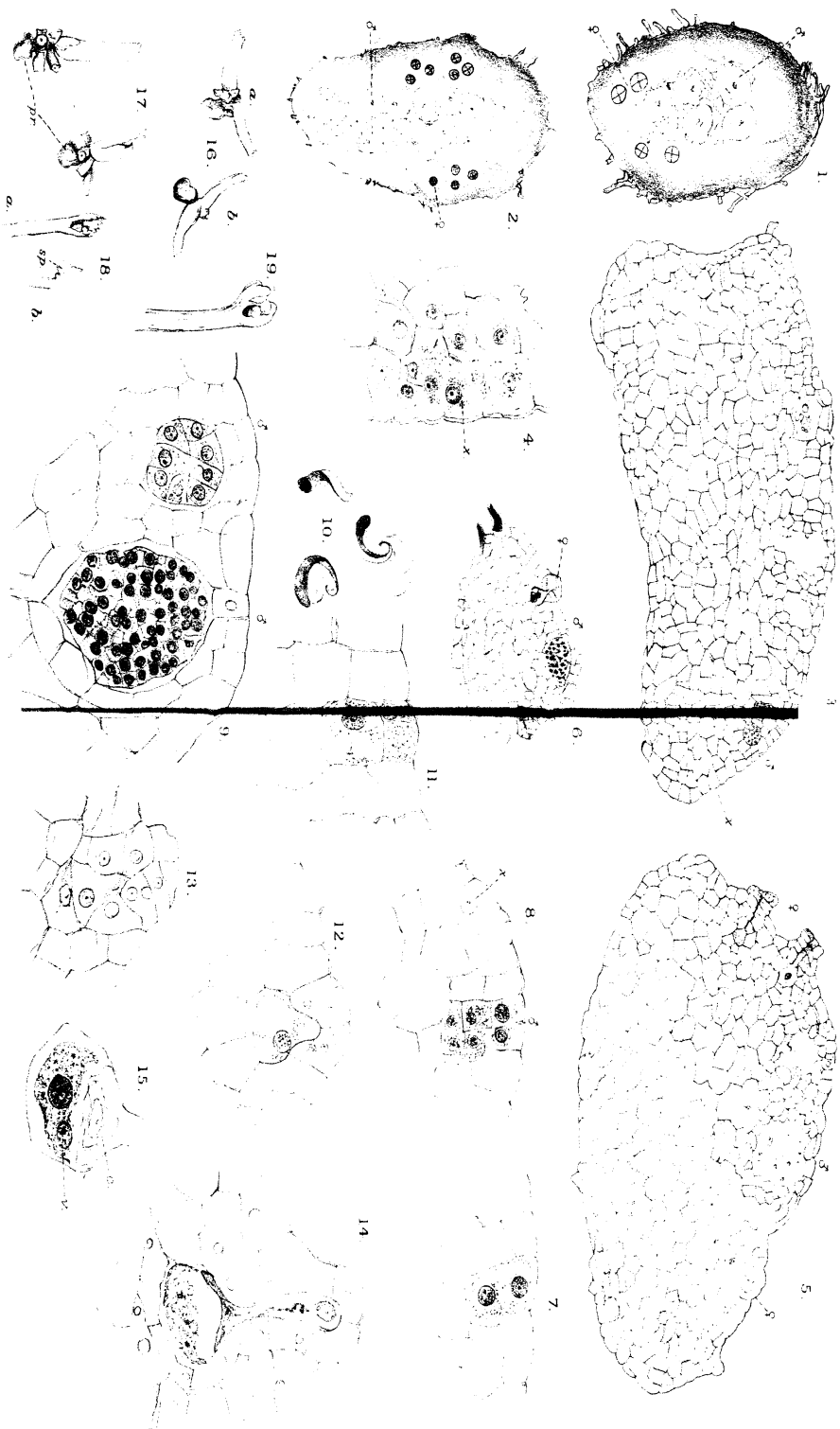
¹ Loc. cit., p. 223.

Dr. Baas-Becking, who has been engaged recently in a study of the later stages of the young sporophyte, finds that *B. simplex* shows some differences in the development of the early leaves, the cotyledon varying somewhat in its development, but never being so reduced as in *B. lunaria*, and there is never such a series of rudimentary leaves as Bruchmann describes for that species. Dr. Baas-Becking suggests that possibly the long subterranean existence of *B. lunaria* in Europe may be due to less favourable conditions than those under which the specimens of *B. simplex* were found in Minnesota. It would be interesting to know how *B. lunaria* in the northern United States and Canada compares in this respect with the European specimens studied by Bruchmann.

EXPLANATION OF FIGURES IN PLATE XVI.

Illustrating Professor Campbell's paper on the Gametophyte and Embryo of *Botrychium simplex*, Hitchcock.

- Fig. 1. Very young gametophyte of *Botrychium simplex*. $\times 50$. δ , antheridia; γ , archegonia.
Fig. 2. A somewhat older gametophyte. $\times 32$.
Fig. 3. Median longitudinal section of a small gametophyte. $\times 100$. x , growing-point.
Fig. 4. Growing-point of the gametophyte. $\times 400$. x , the apical cell.
Fig. 5. Cross-section of the gametophyte, showing the relative position of the antheridia and archegonia. $\times 100$.
Fig. 6. Cross-section of a very small gametophyte. $\times 100$.
Figs. 7-9. Young antheridia. $\times 400$. In Fig. 8, x is the apical cell of the gametophyte.
Fig. 10. Three spermatozoids, from an open antheridium. $\times 900$.
Figs. 11-13. Young archegonia. $\times 400$.
Fig. 14. Median section of a mature archegonium. $\times 400$.
Fig. 15. Venter of a mature archegonium, showing a second nucleus (?), v , perhaps representing a ventral canal-cell; c , lower part of neck canal-cell. $\times$ about 600.
Fig. 16. Two views of a gametophyte with young sporophyte attached. $\times 5$.
Fig. 17. An older sporophyte, still attached to gametophyte, *pr.* $\times 3$.
Fig. 18. a , second leaf with rudimentary sporangiophore. $\times 3$. b , sporangiophore. $\times 10$.
Fig. 19. Second leaf with relatively well developed sporangiophore. $\times$ about 10.



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CAMPBELL — BOTRYCHIIUM.

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Growth and Abscission in Sea Island Cotton.

BY

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With fourteen Figures in the Text.

INTRODUCTION.

THE immediate object of the studies reported in the present paper was to obtain information concerning both the external and the internal factors responsible for the premature shedding of the flower-buds and the young fruit (bolls) of the cotton-plant in St. Vincent. The economic importance of premature abscission is not, of course, confined to the cotton-plant, for it is responsible for considerable losses in a large number of cultivated plants. Future investigation may reveal how far the conclusions arrived at in the course of the present work are of wider application.

Before reporting the results, reference should be made to the conclusions of previous investigators of the problem. The work of Balls (1) in Egypt established a strong presumption that the major factor initiating abscission was a marked water-deficit in the body of the plant. It was found that the elongation of the stem of the cotton-plant was checked immediately the sun struck upon it, and that a slight shrinkage usually followed. A cloud passing across the sun, for instance, was effective in permitting growth, which ceased again as soon as the sun emerged. Balls's conclusion that a net loss of water was the direct cause of growth-inhibition and boll-shedding received confirmation from Lloyd's (6) studies, which were conducted under the relatively humid conditions of Alabama. Ewing's (2) work in Mississippi also indicated that a disturbance in the water-balance of the plant was the main factor responsible for shedding. In St. Vincent, where cotton is probably cultivated under more humid conditions than elsewhere, Harland (3) noted that shedding was heaviest after torrential rain. His observations led him to conclude that root absorption was interfered with as a result of the reduction in the oxygen-

supplying power of the soil, and that consequently a water-shortage ensued, which was the immediate cause of shedding.

MATERIAL.

The work was carried out on the St. Vincent Experiment Station between June 1921 and February 1922. The first series of observations was made on a group of plants which were sown on June 20; this, it may be noted, is approximately the normal time at which cotton is planted in St. Vincent. The second series of observations was made on a group of plants which were not sown till August 27. The environmental conditions which prevailed during the later stages of the development of this group were considerably less humid than in the case of the former.

The June-sown plants which constituted the first group consisted of thirty-five plants distributed at random through a plot of one-tenth of an acre. Two of these were rejected because of damage sustained by wind on September 8. The individual plants in this plot were spaced at intervals of four feet. A daily record was kept for each plant of the rate at which the main axis elongated, of the number of flower-buds and flowers produced on the primary fruiting branches, and of the number of flower-buds and bolls shed. In this group measurements of the length of the main axis were made every day; the number of flower-buds and flowers produced and the numbers shed were not, however, observed on Sunday; these were estimated as far as possible from the observations of the preceding and following days; one-third of Monday's quota was also credited to Sunday; the adoption of this procedure renders caution essential in interpreting the results. The second group was sown on August 27 and consisted of 749 plants, spaced from 18 to 24 inches apart in rows 4 ft. wide. Daily measurements were made of the height of the main axis on thirty-one of these plants, distributed at random throughout the whole population. A daily record was also kept of the number of flowers produced and the bolls shed by all the plants.

THE PROBLEM.

After the work had proceeded a short time, it became apparent that there were three phases of the problem which merited particular attention: (1) the rôle of the physical environment in initiating abscission, (2) the more pronounced tendency to undergo abscission of both flower-buds and bolls produced during the later part of the flowering period, and (3) the tendency for both flower-buds and bolls to be shed when at a very early stage of development.

The graph in Fig. 1 illustrates the latter tendency. The results are based on observations which were made on the first group of plants. The

method adopted in order to determine the age of a flower-bud at shedding requires a word of explanation; the boll presents no difficulties in this respect, for its age can be expressed as the number of days which elapsed between the open flower stage and the completion of abscission. In the case of the flower-bud, however, there is no readily recognizable stage to which its age can be referred. The convention adopted was to express its age at the time of shedding as the number of days which elapsed between the stage in its development which corresponded to the unfolding of the attendant foliage leaf and the completion of abscission; the attendant leaf was judged to be unfolded as soon as the upper surface became clearly visible. The propriety of this procedure is admittedly open to criticism, for it assumes a definite relationship between the development of the bud and

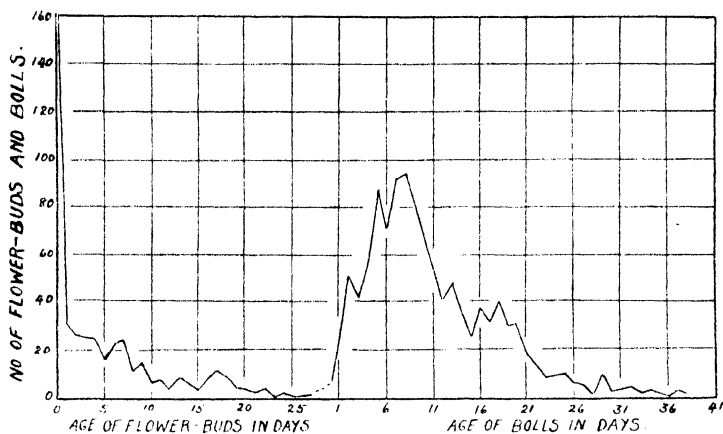


FIG. 1. Age frequencies of flower-buds and bolls of Group I at shedding.

its attendant foliage leaf. It will be observed (Fig. 1) that the majority of flower-buds were apparently shed on the same day that the attendant foliage leaf unfolded. Two hundred and twenty buds were recorded as being shed on this day, whereas only six cases were observed in which the shedding occurred prior to this. There can be little doubt that the explanation of these observations lies in the fact that a large number of flower-buds were shed before the foliage leaf expanded, but escaped notice owing to their minute size. It may be remarked, however, that a careful inspection will generally reveal the presence of the flower-bud from three to five days preceding the unfolding of the leaf.

It is clear (Fig. 1) that there is a very marked tendency for the flower-buds to be shed when very young, and that they become progressively less liable to suffer abscission as they grow more mature. It is of some interest to observe (Fig. 2) that this tendency becomes more pronounced as the development of the plant advances.

Referring again to Fig. 1, it will be noted that another period of marked susceptibility occurs shortly after anthesis, the vast majority of the

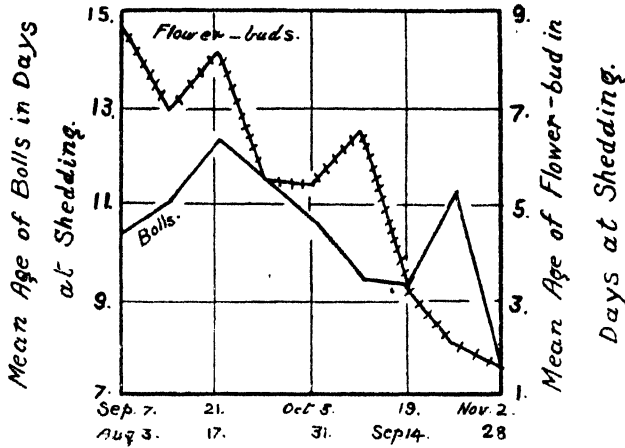


FIG. 2. Showing weekly changes in mean age in days of flower-buds and bolls of Gp. I at shedding.

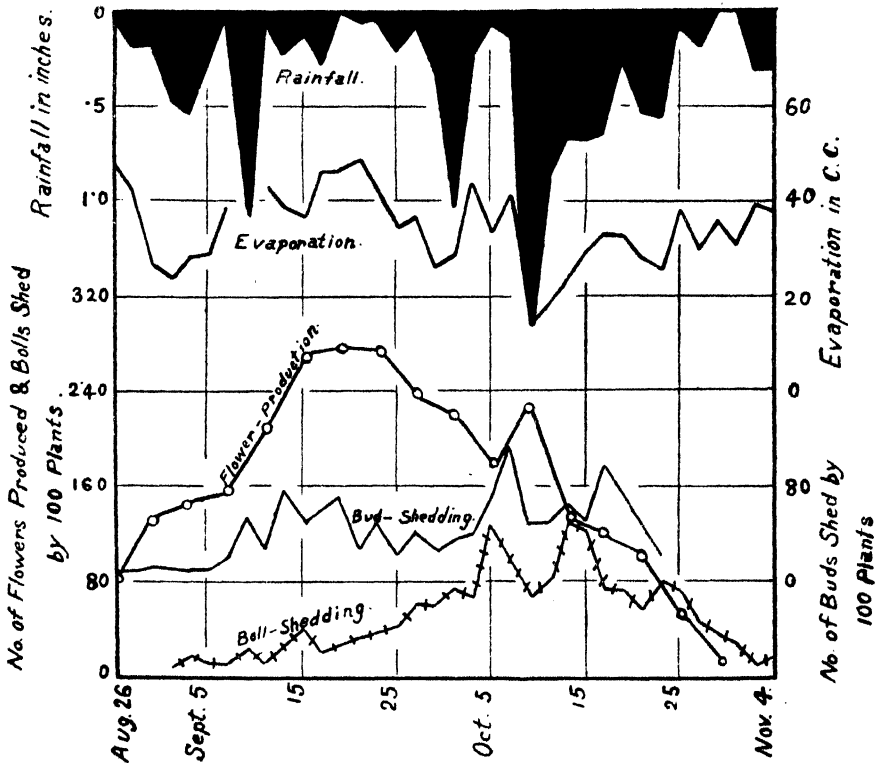


FIG. 3. Rates of flower-bud and boll-shedding, flower production, evaporation, and rainfall. Gp. I.

bolls being shed when from five to nine days old. A large proportion of the more mature bolls, when shed, were affected with external boll disease (*Pseudomonas Malvaccarum*). It has, of course, long been recognized that both bacterial and fungous diseases of the boll and insect punctures, or any other factor liable to cause injury, increase the susceptibility to shedding.

An indication of the part played by the physical environment in initiating abscission, and the more pronounced tendency of the flower-buds and bolls produced during the later part of the flowering period to undergo

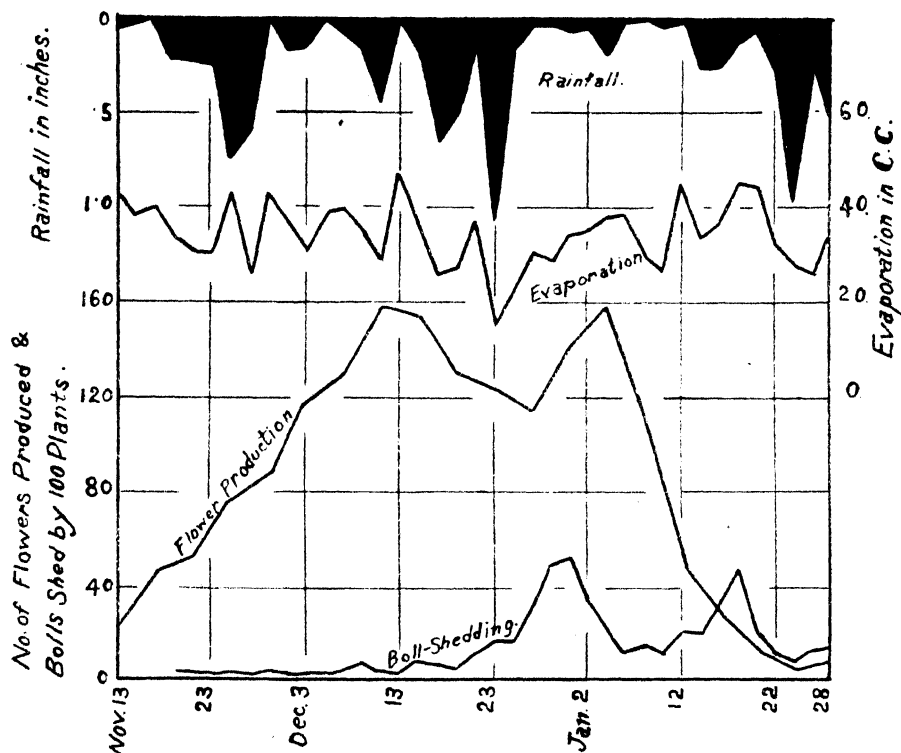


FIG. 4. Rates of boll-shedding, flower production, evaporation, and rainfall. Gp. II.

abscission, can be obtained from the graphs in Figs. 3 and 4. The daily rates of flower production and boll-shedding are exhibited for both groups, but of bud-shedding only for the first group. For comparison with these shedding rates, the evaporation from the Livingston (5) spherical black atmometer and the rainfall have also been inserted. The daily rates of flower production have been smoothed to four-day means, and the shedding rates, evaporation, and rainfall to two-day periods. Attention should be drawn to the small proportion of bolls shed during the earlier portion of the flowering period. The results indicate that quite a small stimulus during the later part of the flowering period may result in a relatively large

proportion of flower-buds and bolls being shed, and that a stimulus of equal intensity occurring during the preceding period may lead to little or no shedding.

Concerning the nature of the environmental factors which are to be associated with the modes in the shedding curves only the briefest mention is necessary at this stage. The waves of boll-shedding, it may be observed, are generally, but not always, preceded by periods of considerable precipitation and low rates of evaporation. Periods of heavy rainfall have, of course, long been associated in the mind of the West Indian cotton-grower with extensive waves of flower-bud and boll-shedding. It will be seen that the curves of flower-bud- and boll-shedding tend to synchronize. The error introduced into the results for bud-shedding by omitting to reckon a large number of flower-buds until the opening of the foliage leaf excludes the possibility of any close correspondence between the two. With this brief introduction, it is possible to proceed with the analysis of the problem.

SUSCEPTIBILITY TO SHEDDING.

The internal factors responsible for the marked susceptibility to shedding which is noticeable during the later portion of the flowering period have apparently received in the past little or no attention. Balls (1), however, recognized two causes, constitutional and environmental, at work in effecting shedding. Lloyd (6), on the other hand, though emphasizing the fact that a progressive increase occurred in the proportion of flowers which were subsequently shed, ascribed this change to the gradual reduction in the moisture content of the deeper soil-layers. 'Shedding', he says, 'is always a response to untoward conditions.'

The lower graphs in Fig. 5 represent the approximate percentage of flowers produced on any day which were subsequently shed; they were obtained from the observed rates of flowering and of boll-shedding by assuming that a period of ten days elapsed between the open flower stage and the completion of abscission; ten days was, as a matter of fact, the mean period in the first group; the records of the second group do not, however, permit of an exact estimate being formed. As the results are smoothed to four-day intervals the error introduced by this assumption is not of grave consequence.

The mean daily growth-rates of the main axis of both groups have been smoothed for a similar period and are also reproduced in Fig. 5. Inspection of the two sets of graphs reveals the fact that the percentage of flowers which subsequently underwent abscission remained relatively insignificant until growth of the main axis had almost ceased. It is difficult to escape the conclusion that the fluctuations in the physical environment which generally herald waves of shedding are not of marked

importance in initiating abscission until after the inhibition of growth in the main axis.

The results of a recent investigation (7) on correlation in the cotton plant is of some interest in this connexion. In the course of that work it was found that the growth-rate of the main axis commenced to decline as soon as boll-development was initiated, and, moreover, that this retardation in the growth-rate could be deferred by suppressing fruit development. It was also demonstrated that growth-cessation in the main axis was not due to any autogenic change within the terminal meristem, for renewed activity of the apparently senescent apical bud occurred after it was isolated and budded on a young plant.

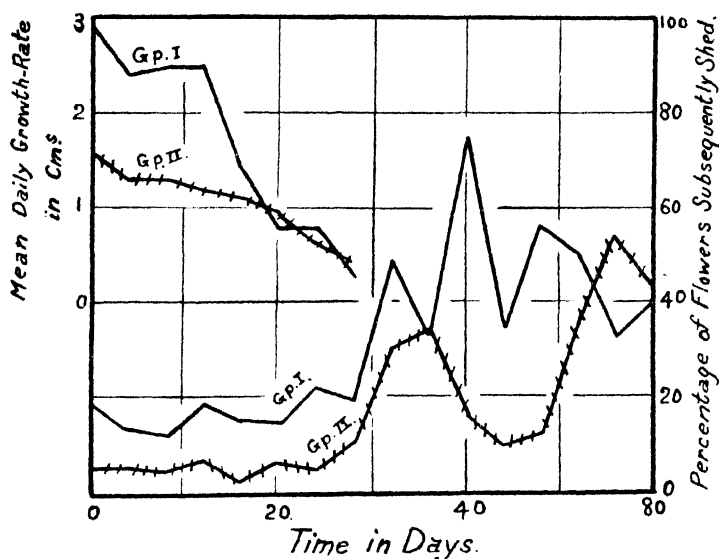


FIG. 5. Percentage of flowers produced daily which were subsequently shed, and daily growth-rates of main axis. Gps. I and II.

As a result of these and other observations, which need not be repeated here, it was concluded that the decline in the activity of the apical meristem was due to a correlation factor, which was introduced by the development of fruit on the basal fruiting branches. It was suggested that it was in some way to be associated with a deflexion of growth-promoting substances, especially carbohydrates, from the apical to the basal part of the plant.

It seems plausible to infer that the greater liability of bolls, produced after growth-cessation has taken place in the main axis, to undergo abscission may also be associated with a deflexion of assimilates from the apical to the basal fruiting branches, for the major part of the fruit produced during the latter part of the flowering period is, of course, situated on the apical part of the plant. Should this assumption be warranted, it would

follow that the incidence of any external growth-retarding factor would lead to a check in the rate at which elaborated food was being produced, and the consequent reduction in the supply of assimilates might well furnish, either directly or indirectly, the stimulus for augmented rates of shedding.

The hypothesis is supported by an experiment made by Ewing (2) in Mississippi, in which it was found that the daily removal of all the flowers almost doubled the amount of flowering, but that the destruction of one-half of the flowers produced during the last six-tenths of the flowering period led to a scarcely appreciable increase in the flower production; it did, however, almost completely offset the natural tendency to shedding. Presumably the supply of assimilates liberated as a result of the suppression of the fruit development is expended in further flower production, but in the presence of a number of growing bolls any assimilates available are mainly employed in furthering their development, and thereby reducing their liability to be shed. The shedding of a certain proportion of the flower-buds and young bolls is probably inevitable, and need not, therefore, be viewed with concern.

ENVIRONMENTAL FACTORS AND BOLL-SHEDDING.

The difficulties encountered when the attempt is made to trace a quantitative relationship between the percentage of young bolls eliminated by abscission and the causative external factors, arise on the one hand from the complexity of the processes which determine abscission in the plant body, and on the other from our inability, as yet, to measure many of the environmental factors which directly influence the metabolism of the plant. To these difficulties must be added those introduced by the biological environment, fungous and bacterial diseases, and insect depredations.

The rates at which boll-shedding occurred during the later portion of the flowering period are shown for each of the groups in Figs. 6 and 7 respectively. In the first group, it will be remembered that the number of bolls which were shed on Sundays was not directly determined, and that one-third of Monday's quota was credited to these days, which are indicated on the graphs by the solid black circles. A word of explanation is also needed concerning the environmental factors of which records were kept, and the method of their presentation.

Evaporation from the Livingston spherical atmometers, both black and white, was measured daily in duplicate at 9 a.m.; the difference is represented in the figures as 'Evaporation due to solar radiation', whereas 'Evaporation' refers to the total evaporation from the black atmometer. 'Daytime rain' indicates the amount of precipitation which occurred between 9 a.m. and 3 p.m.; it therefore represents the rainfall occurring during the hottest part of the day. A daily record of the temperature, both of the subterranean and aerial environments, was also kept, but, owing to the small

amount of variation and the apparent absence of any significant relationship, it has not been reproduced.

The environmental factors have been displaced five days to the left in each of the figures. Such a procedure, of course, assumes that a constant interval of approximately five days intervenes between the incidence of the causative factor and the completion of abscission. It was found, however, that by doing this a reasonably close correspondence could be traced between certain external factors and the major waves of boll-shedding. Ewing's studies, too, it may be noted, indicated that five to six days represented

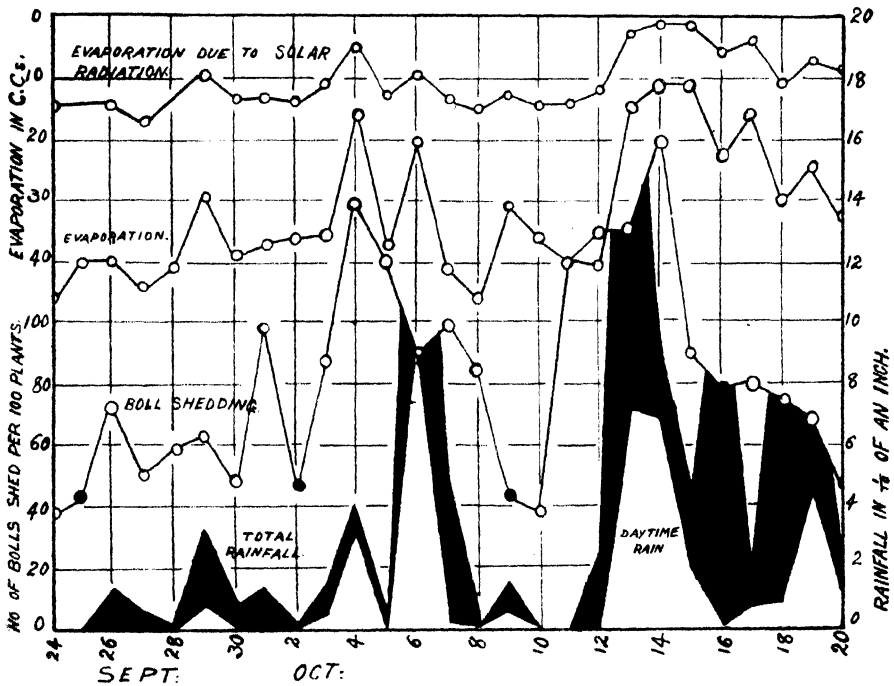


FIG. 6. Daily rates of boll-shedding, rainfall, evaporation, and evaporation due to solar radiation. Gp. I.

under natural conditions the mean interval between the stimulus and the abscission response, while Lloyd's work in Alabama pointed to an approximately similar period.

Reference to Fig. 4 will show that the two main waves of shedding (commencing October 2nd and 10th respectively) were both preceded by periods of heavy daytime rain and low rates of evaporation. To the minor waves, commencing October 26, no significance can be attached, for the probability exists that they would have been eliminated had the population been larger. In Fig. 7, for instance, which shows the results for the 749 plants of the second group, these minor irregularities are almost

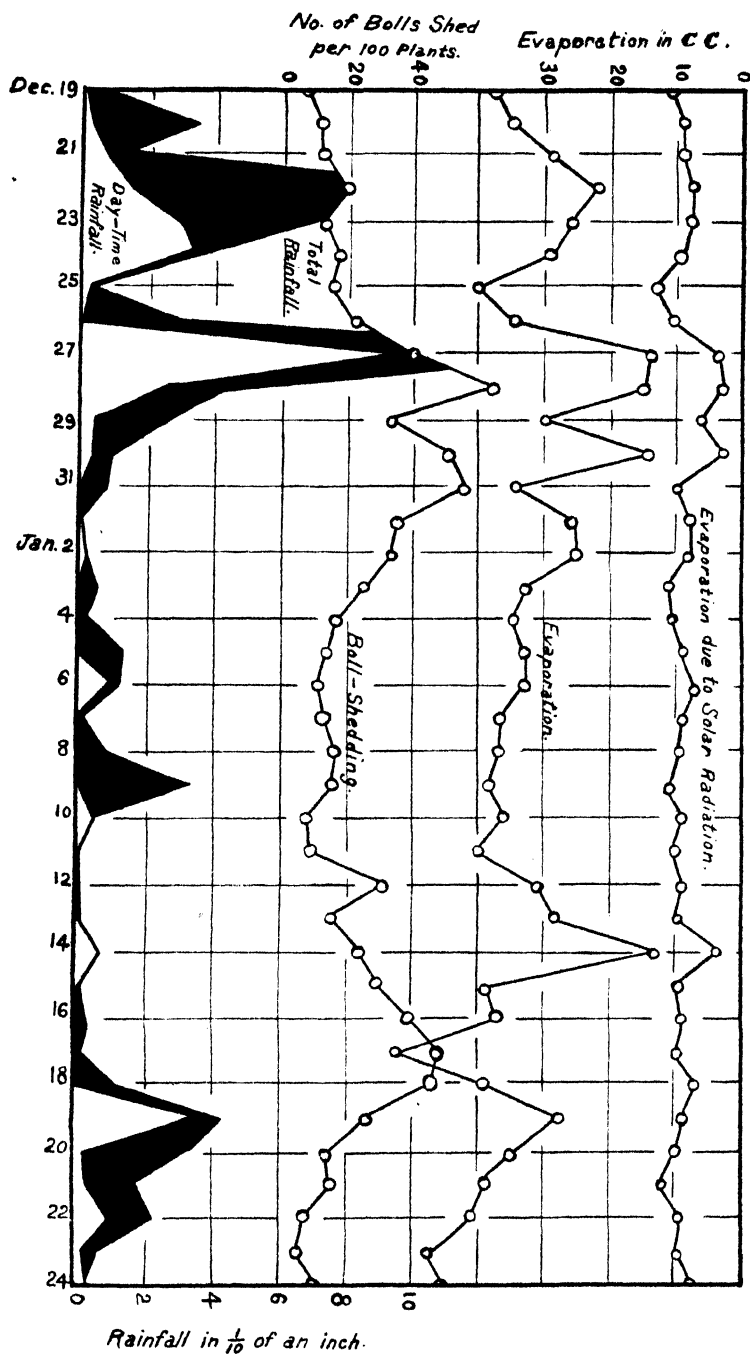


FIG. 7. Daily rates of boll-shedding, rainfall, evaporation, and evaporation due to solar radiation. Gp. II.

completely removed. Here, too, it will be seen, the first shedding wave (commencing December 26) followed five days after a period of heavy daytime rain and low rates of evaporation. The second wave, it will be noted, followed some five days after a change from very low to rather high evaporation rates. It will be remembered, however, that the probability exists that a very slight stimulus during this later portion of the flowering period may initiate shedding, and that even in the absence of a stimulus of any sort a certain amount of shedding is apparently inevitable.

↳ To sum up, the results suggest that heavy rainfall is not directly responsible for the augmented rates of shedding; nor yet, it would seem, is daytime rain to be especially associated with the initiation of abscission. It will be observed, however, that daytime rain when accompanied by very low evaporation rates is followed by pronounced shedding. That daytime rain may have occasioned some of the shedding as a result of the destruction of pollen is very probable. A more fundamental cause than the absence of pollination must, however, be sought. For it cannot but be significant, as Harland (3) has pointed out, and as the results presented in the next section indicate, that the waves of bud- and boll-shedding tend to be synchronous. Moreover, the relatively small percentage of flowers which subsequently underwent abscission, in spite of daytime rain, during the first half of the flowering period clearly indicates how slight is the part played by this factor in bringing about shedding.]

THE GROWTH-RATE OF THE MAIN AXIS AND ENVIRONMENTAL FACTORS.

In order to determine how the growth-rate of the main axis was influenced by daytime rain and low rates of evaporation, which the results presented in the preceding section seemed to suggest as being the precursors of the augmented rates of boll-shedding, the growth-rate of the thirty-three plants which constituted the first group was determined daily, between 9 and 11 a.m., until growth became so small that no significance could be attached to the daily variations. The measurements were made from the cotyledonary node to the terminal bud. In Fig. 8 the mean results of these measurements for a period of forty days are presented for comparison on the one hand with rainfall and evaporation, and on the other with the rates of bud- and boll-shedding. Inasmuch as some of the flower-buds were shed before the opening of the attendant foliage leaf, and consequently the day on which they were shed is not known, only those which were shed subsequently have been included in the results presented in Fig. 8.

Inspection of the graphs betrays the somewhat remarkable fact that low rates of evaporation accompanied, as is generally the case, by daytime rain corresponded with a marked retardation in the growth-rate. The amount of evaporation on September 8 and 9 was not determined, as the

atmometers were dislodged by the wind ; evaporation was, however, as may be inferred from the amount of daytime rain, very small on the 8th, and also on the 9th. The tardiness in the rates of recovery of the growth-rate at this time was partially due, no doubt, to root injury sustained as a result of the wind, but was mainly, as reference to Fig. 4 indicates, due to the normal decline in the growth-rate occasioned by the augmentation in the number of the developing fruits. This conclusion is sustained by the rapidity with which some young plants in a neighbouring plot recovered. The plants were not measured on the 8th, so that the growth-rates for this and the following day have been taken as the mean of the two-day period.

Referring again to Fig. 8, it will be seen that a wave of bud-shedding occurred 4-5 days, and of boll-shedding 5-6 days after September 8. The shedding waves which took place on the 7th, 8th, and 9th of the same month commenced six days later than the retardation in the growth-rate of the main axis which occurred on the 1st. The comparative absence of shedding during the earlier part of the period is not only ascribable to the paucity in the number of flower-buds and bolls, but also to their very slight liability to undertake the abscission response at this period.

It will be evident that no definite relationship can be traced between the daily fluctuations in the growth-rate of the main axis and the amount of shedding which ensued. The results, nevertheless, indicate a strong probability that the same external factors—to wit, daytime rain and low rates of evaporation—which cause a retardation in the growth-rate during the earlier periods of the plant development, are also responsible for the extensive shedding of bolls which occurs during the later portion of the flowering period. It would follow, if this view is correct, that the same internal factors are the cause of both phenomena. As yet the relative importance of these various internal factors cannot be diagnosed, for daytime rain and the reduction in light intensity which accompanies low rates of evaporation might retard not only the rates of carbon assimilation, but would also lead to large turgor pressures (possibly attended by incipient guttation), which would limit the supply of electrolytes normally translocated with the transpiration current ; the net result would presumably be a check in the supply of elaborated food. It is also conceivable that the translocation of assimilates might be inhibited by the augmentation in the turgor pressure.

The difficulty encountered when the attempt is made to ascertain whether or no the extent of the daily fluctuations in the growth-rate is directly related to the amount of boll-shedding arises of course from the fact that throughout the period of greatest susceptibility to shedding the growth of the main axis has practically ceased. At first sight it might appear feasible to bridge this gap by determining whether the amount of

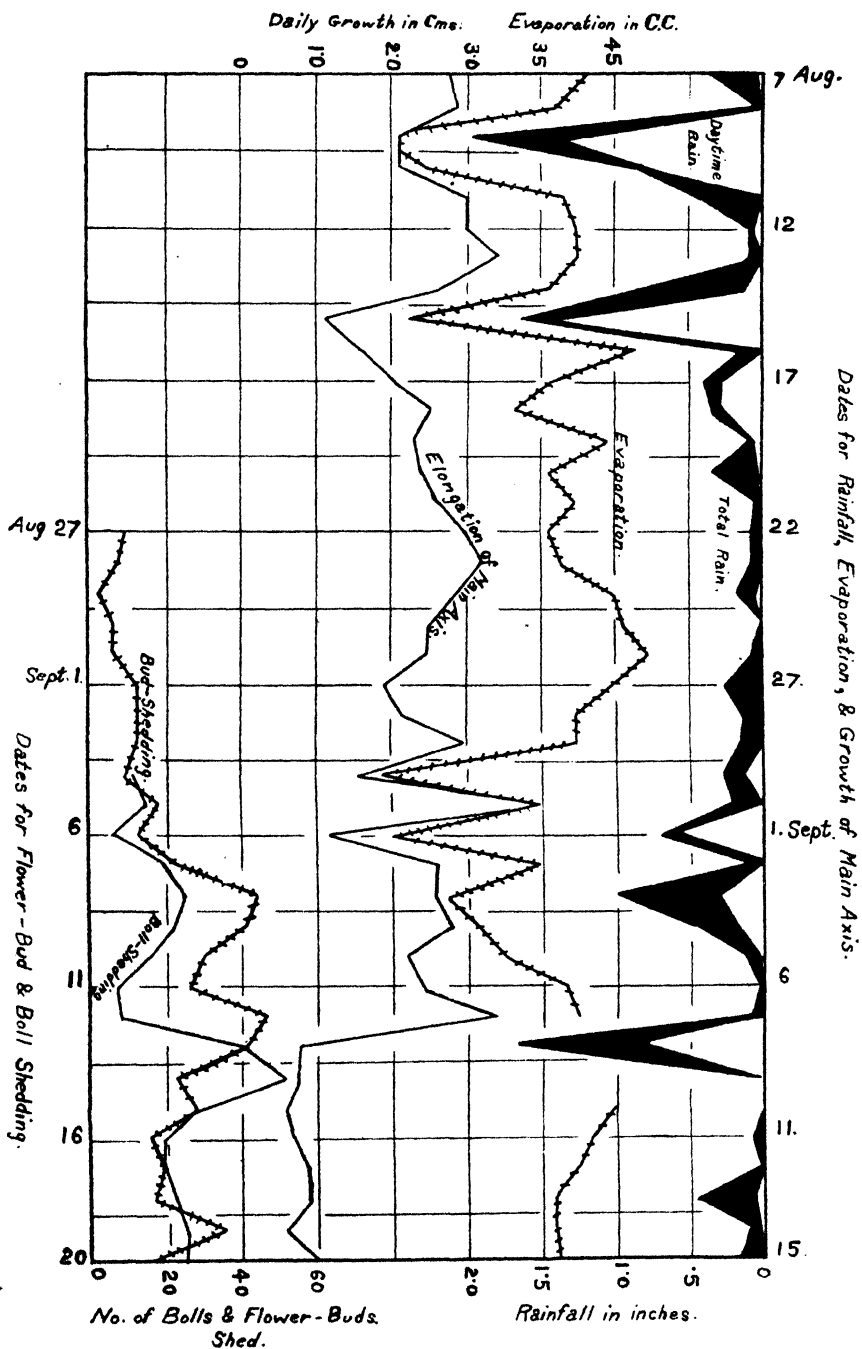


Fig. 8. Daily rates of flower-bud and boll-shedding, axis elongation, evaporation, and rainfall. Gp. I.

variability in the daily growth-rates of the individual plants and the percentage of buds and bolls shed in the same period were correlated or not. The smallness of the population and the very limited amount of shedding which occurred while growth was in progress must render such a procedure very dubious. Nor is it possible to obtain a single index which will express the amount of variation occurring in the growth-rate on consecutive days, for the coefficients of variability, it will be clear, would also express the amount of variation over more extended periods.

THE GROWTH-RATE OF THE MAIN AXIS AND THE SUBTERRANEAN ENVIRONMENT.

Though the results presented in the previous section seemed to render it improbable that the subterranean environment could play any considerable part in causing the growth-retardation which occurred during periods of daytime rain and low evaporation, it seemed none the less desirable to attempt to place the matter on an experimental basis. The growth-rates of four groups, each of ten plants, were accordingly determined daily. The plants were not quite twelve weeks of age when the measurements recorded in Fig. 9 were undertaken. With a view to avoiding the decline in the growth-rate which attends fruit development, all the bolls were pruned off some days previously and the flowers removed daily as they were produced. Drains about one foot in depth were made on each side of the third and fourth groups in order to ensure against the oxygen-supplying power of the soil being markedly diminished during periods of excessive rainfall. Inasmuch, however, as rain is a saturated solution of oxygen and the soil of St. Vincent is extremely permeable, the possibility of such a contingency must be regarded as remote. To the first group approximately 10,000 c.c. of water, to which 2 c.c. of a 3 per cent. solution of hydrogen peroxide were added, were applied daily to the soil round each of the plants from October 17 until the end of the experiment. On October 16 a sheet of white linoleum extending about three feet on each side of the plants was placed over the surface of the soil in the neighbourhood of the fourth group. The space between the linoleum and the plants was sealed with wax; the subterranean environment of this group was thus isolated from the direct effects of heavy rain.

Inspection of the graphs in Fig. 9 reveals the remarkable fact that growth was apparently entirely suspended on October 9 in every group. The individual records showed an extreme range of from -0.8 to 1.0 cm. The measurements on the 9th were, however, made after midday, and not, owing to the torrential rain, which fell in the early part of the day, at 8 a.m. as usual. The net result of this delayed measurement may have been to diminish somewhat the growth credited to the 9th, and to have augmented that of the 8th.

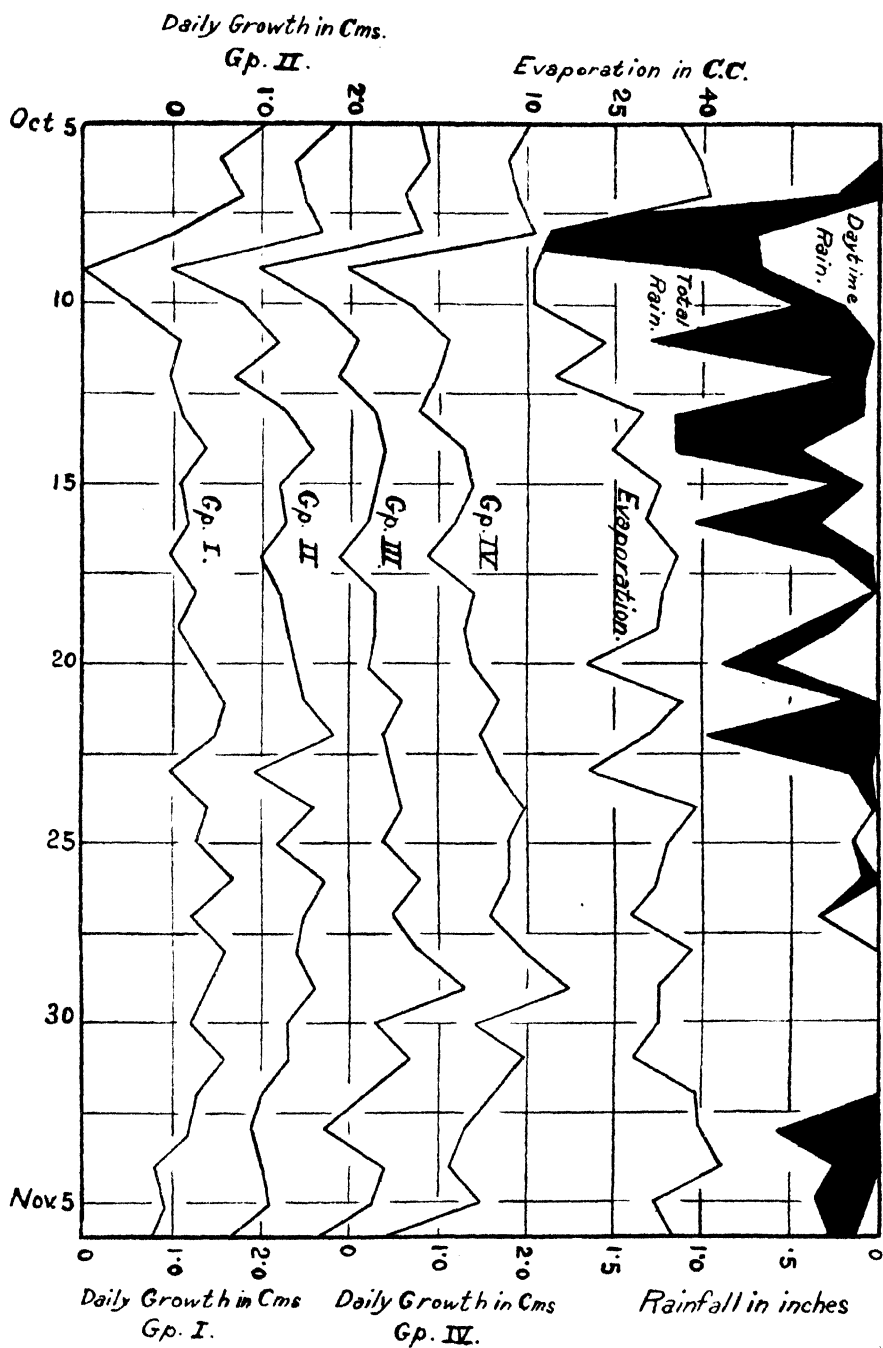


FIG. 9. Daily growth-rate of four groups of plants. Evaporation and rainfall. Gp. I. Not drained. 10,000 c.c. of water, 2 c.c. of 3 per cent. H_2O_2 added daily from October 17. Gp. II. Not drained. Gp. III. Drained. Gp. IV. Drained. Linoleum sheet placed on soil on October 16.

It is important to observe that no relationship can be traced between the fluctuations in the daily rainfall and the rate of growth. The only marked check in the growth-rate occurred during a period of low evaporation which was as usual accompanied by daytime rain and little or no direct solar radiation. It cannot but be considered remarkable that the heavy daily application of water to Gp. I, and the isolation of the subterranean environment in Gp. IV, should have been without any appreciable influence on the daily growth-rate. On the whole, the results confirm the view that inhibition of growth in the main axis is due to a change in the aerial rather than in the subterranean environment.

WATER RELATIONS AND DAYTIME GROWTH.

Reference has already been made to the fact that every previous investigation of the factors responsible for abscission has pointed to the importance of the plant's water relations. Balls's work, for instance, leaves no scope for doubt that under Egyptian conditions a pronounced water-deficit in the plant body is the main cause of shedding. He demonstrated that in the sunshine the growth of the cotton-plant is inhibited. This growth-inhibition while the sun was up he called the 'sunshine effect'. He showed that it resulted from the increased tension due to the excessive rates of transpiration by shading a plant with a bell-jar, whereupon growth was resumed even in the sunlight. Lloyd demonstrated that precisely the same relation was shown by the cotton-plant in Alabama, where a cessation of growth or shrinkage during a portion of the hours of sunlight was noted. The results of the experiments recorded in the preceding sections suggest, however, that the growth-inhibition which precedes shedding in St. Vincent results from a check in the assimilatory activity of the leaves rather than from marked daily fluctuations in the aridity of the plant's environment. It may be stated, however, that one of the results of a marked daily water-deficit would be to close the stomata and thus inhibit carbon assimilation.

In order to ascertain whether the 'sunshine effect' occurred under the very humid conditions under which cotton is grown in St. Vincent, the thirty-one plants of the second group were measured both shortly after dawn (6 a.m.—8 a.m.) and also towards dusk (4 p.m.—6 p.m.); the daytime interval between the measurements being therefore approximately 10 hrs. With a view to indicating how the growth during the day compared with that at night, the observed increments in growth have been expressed on a 24-hour basis. The results, which have been smoothed to four-day periods, are shown graphically in Fig. 10. The daily evaporation and rainfall, smoothed for a similar period, are also reproduced in the figure. Inspection of the graph discloses the fact that growth was inhibited to only a very small extent during the hours of sunshine. Some retardation in the

growth-rate is inevitable as a result of the augmented water strain which attends the more rapid ascent of sap during the hours of sunlight. The ratio between the day and the night growth-rate is also shown in the figure; this is of interest in that it demonstrates that the daytime growth-retardation

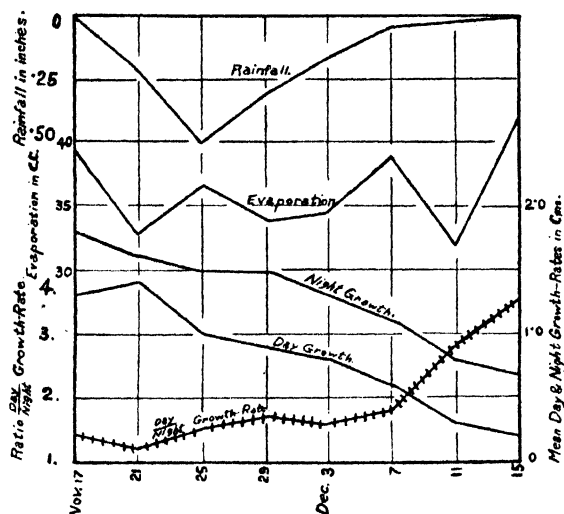


FIG. 10. Day and night growth-rates brought to a 24 hour basis, ratio $\frac{\text{day}}{\text{night}}$ growth-rate, evaporation, and rainfall. Gp. II.

became more marked as the daily growth-rate approached zero. This is possibly the result of a decrease in the water-supplying power of the soil, but the operation of internal factors is not excluded. In view of these results it becomes comprehensible why, under the conditions in St. Vincent, little or no shedding can be traced to pronounced saturation-deficits in the plant body.

THE GROWTH-RATE OF THE BOLL AND FERTILIZATION.

In an earlier section it was pointed out that the young boll is especially liable to be shed when about seven days of age. This in the past has sometimes been considered indicative of a factor inhibiting fertilization (1). It would seem impossible normally to associate this with unsuccessful pollination, for the interval elapsing between the reception of the stimulus and the completion of abscission is under field conditions only about five days. Moreover, it will be remembered that the more constant cause of shedding appeared to be associated with conditions which tended to retard the growth of the plant as a whole rather than with the influence of daytime rain on individual flowers. It seems legitimate to infer that the metabolic transformations which precede abscission, and which probably in

some way inhibit fertilization, or, what is more probable, inhibit the stimulus to fruit development, are especially liable to be initiated some days after rather than during the open flower stage. The results of some measurements of the growth-rate of the bell, which seem to bear on the point, will now be considered.

The two graphs in Fig. 11 represent the daily growth-rates of forty-two bolls, thirty-eight of which completed development and four of which were shed on the seventh and eighth days after the open flower stage. The measurements were made at 10 a.m. daily, and represent the daily rate of increase in the diameter of the boll in thirty-sixths of an inch (0.71 mm.).

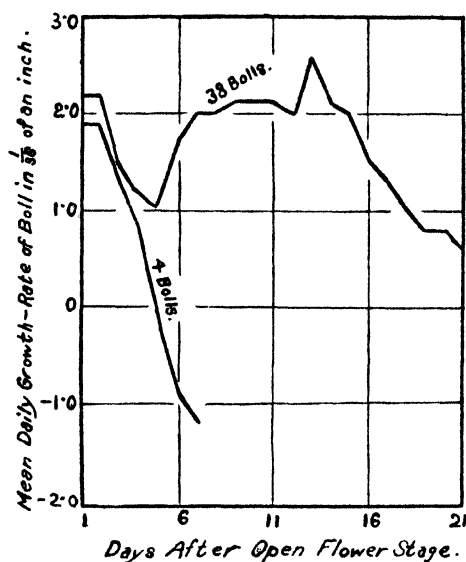


FIG. 11. Showing the two stages in the growth of a boll.

The growth of the thirty-eight bolls which completed development, it will be seen, falls into two distinct stages. The first, which terminated on the 5th day, is one of increasing growth, whereas the second stage, which follows it, is suggestive of the curve of autocatalysis, that is to say that the rate is initially slow, increases, and then declines. Comparison with the growth of the four bolls which were shed on the 7th and 8th days shows that the first stage is essentially similar in both, but that in the latter recovery failed to occur. It is certainly very suggestive that the period which other considerations point to as the period of greatest

susceptibility to the stimulus which initiates abscission should be also one of growth-retardation. It will be remembered that the period of greatest susceptibility to shedding did not occur until after the inhibition of growth in the main axis, and that the same external factors which were associated with a retardation in the growth-rate of the main axis also preceded augmented rates of shedding.

The next point which calls for comment is the rôle played by fertilization. Balls, it will be recalled, has pointed out that fertilization in the cotton-plant is normally completed within thirty hours after the opening of the flower, i. e. by the afternoon of the following day. If recovery in the growth-rate on the fifth or six days after the open flower stage is dependent on fertilization, it is an interesting fact that a delay of 4-5 days should occur between the completion of fertilization and the transmission of the

stimulus to fruit development. In order to ascertain whether this was so or not, two lots, each of twenty-five flowers, were tagged. The stamens were removed from both lots on the day preceding the opening of the flower. On the following day one lot was pollinated and the corollas of both lots were closed with string. All the bolls which had been pollinated

completed development, whereas twenty-two of the non-pollinated lot were subsequently shed. The three bolls of the latter lot which were not shed appeared to have normally developed seeds, and therefore presumably had been pollinated, possibly by ants or thrips entering at the base of the flower between the petals. Inspection of the graphs in Fig. 12 will show that recovery from the initial decline in the growth-rate is dependent on the stimulus communicated by fertilization, for though only thirteen of the bolls which were shed are represented in the figure, yet none showed any indication of recovery. The conclusion seems justified that the pronounced liability of the boll to be shed when about seven days old is associated with the growth-retardation which occurs for some days following the open flower stage, and that this in turn is due to the lag

which occurs between the completion of fertilization and the communication of the stimulus for fruit development. The metabolic transformations which precede abscission are apparently initiated during this critical period of growth-retardation, and doubtless in some way inhibit the stimulus to augmented growth arising from the process of fertilization. The tendency of the flower-buds to be shed at a very early stage of development is conceivably also to be attributed to the slow rate of growth at that time, though no direct evidence in support of this view is obtainable. It will be observed that the assumption has been made that the growth-rate is an index to the rate of metabolism or physiological activity, and that low rates of metabolism render the flower-bud or boll especially liable to undergo abscission. A more extended discussion of this point will be deferred until the results of some additional experiments have been presented.

ABSCISSION AND THE SUPPLY OF ASSIMILATES.

The ages of two lots of bolls at shedding, one of 25 and the other of 30, are shown in Table I. The growth-rates of 13 of the first lot were considered in the preceding paragraph (Fig. 12). Pollination was prevented in both lots by the removal of the stamens on the day prior to flowering.

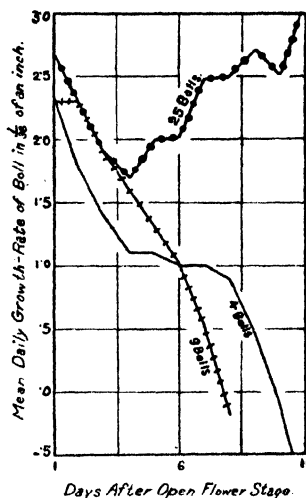


FIG. 12. Showing recovery in the growth-rate of the boll due to stimulus of fertilization.

It will be seen that the age of the first lot at shedding was markedly greater than that of the second, which in this respect was just about normal. The question which presents itself is, Why should the completion of abscission have been delayed to such an extent in the first lot? for the stimulus to abscission, in the absence of fertilization, must have come into operation at the same time in both lots. There is little doubt that the explanation lies in the fact that the bolls of the first lot belonged to plants from which all the fruit had been removed, thus augmenting the normal supply of assimilates, whereas the bolls of the second lot belonged to an ordinary field population, which had received no treatment of any kind. The inference to be drawn from this experiment is presumably that the interval elapsing between the occurrence of the factor initiating abscission and the completion of the process is determined in a large measure by the supply of assimilates available.

TABLE I. *Number of Bolls shed on Successive Days after Open Flower Stage.*

<i>Days after open flower stage.</i>	<i>Lot I. Bolls, Flowers and other bolls removed.</i>	<i>Day after open flower stage.</i>	<i>Lot II. 30 bolls.</i>
9	4	3	1
10	5	4	—
11	2	5	1
12	2	6	5
13	4	7	10
14	—	8	8
15	—	9	4
16	2	10	1
17	1	11	—
18	—	12	—
19	—	13	—
20	2	14	—

In the course of the preceding pages, it will be recalled, the hypothesis was advanced that the progressive increase in the susceptibility to shedding resulted from the fact that the rate at which food is elaborated by the plant tends to lag behind the rate at which it is utilized by the developing fruit, and that any retardation in the rate of food elaboration results in augmented rates of shedding. Professor Farmer suggested that it might be instructive to observe the effect on shedding of cutting off the supply of assimilates. Exposure of plants at different stages of development to an atmosphere devoid of carbon dioxide for definite periods would have been the most satisfactory method of doing that, but the experiment was not found to be practicable; removal of the foliage leaves was accordingly resorted to. The plants selected for the experiment had at the time a number of maturing bolls. A number of flowers (331) were tagged on three successive days and the leaves removed from approximately half the plants on the fourth day. Accordingly, at the time of the operation, the bolls ranged from one

to four days in age. The results, which are recorded in Table II, show that 96.5 per cent. of the bolls were shed within nine days from the plants which had been deprived of their leaves, and that only 39.6 per cent. were lost by the control plants in the same period. Of great importance is the fact that the maximum rate of shedding occurred on the fifth day after the operation; the period intervening between the application of the stimulus and the completion of abscission was thus similar to that occurring under natural conditions. The rapidity and completeness of the response can only indicate, moreover, that the reserve food substances in the cotton-plant quickly become exhausted.

TABLE II. *Number of Bolls shed on Successive Days after Open Flower Stage.*

Days after open flower stage on which shedding occurred	1	2	3	4	5	6	7	8	9
172 Bolls. Leaves removed	1	4	13	22	47	38	29	11	1
159 Bolls. Leaves not removed	1	2	5		6	14	16	10	6

THE GROWTH-RATE OF THE BOLL AND ENVIRONMENTAL FACTORS.

The retardation in the growth-rate of the main axis, which occurred during periods of low light intensity, low evaporation, and daytime rain, conditions which it will be recalled were also followed by augmented rates of shedding, suggested the possibility that these conditions might also be recorded by a check in the growth-rate of the boll.

The growth-rate of three lots of bolls, none of which was shed, are shown in Fig. 13. There were twenty-one bolls in the first lot, thirty-eight in the second, and forty-six in the third. The results represent the mean growth-rates for each lot. Evaporation and rainfall are also shown for comparison with the growth-rates. The environmental conditions which prevailed on October 8th, 9th, and 10th were possibly responsible for the fact that recovery in the growth-rate of the first lot did not take place until the seventh day after the open flower stage. Moreover, it may not be a coincidence that the decline in the growth-rate of the second lot was particularly marked during the same period. It will also be observed that the growth-retardation following anthesis was less pronounced in the third lot than in the first two, and that this synchronized with higher rates of evaporation and only a relatively small amount of daytime rain. It is rather remarkable that the growth-rates of all the three lots should have been greatly augmented on October 18. The results, though not decisive, nevertheless suggest that the same external conditions which retard the growth of the main axis also depress that of the boll.

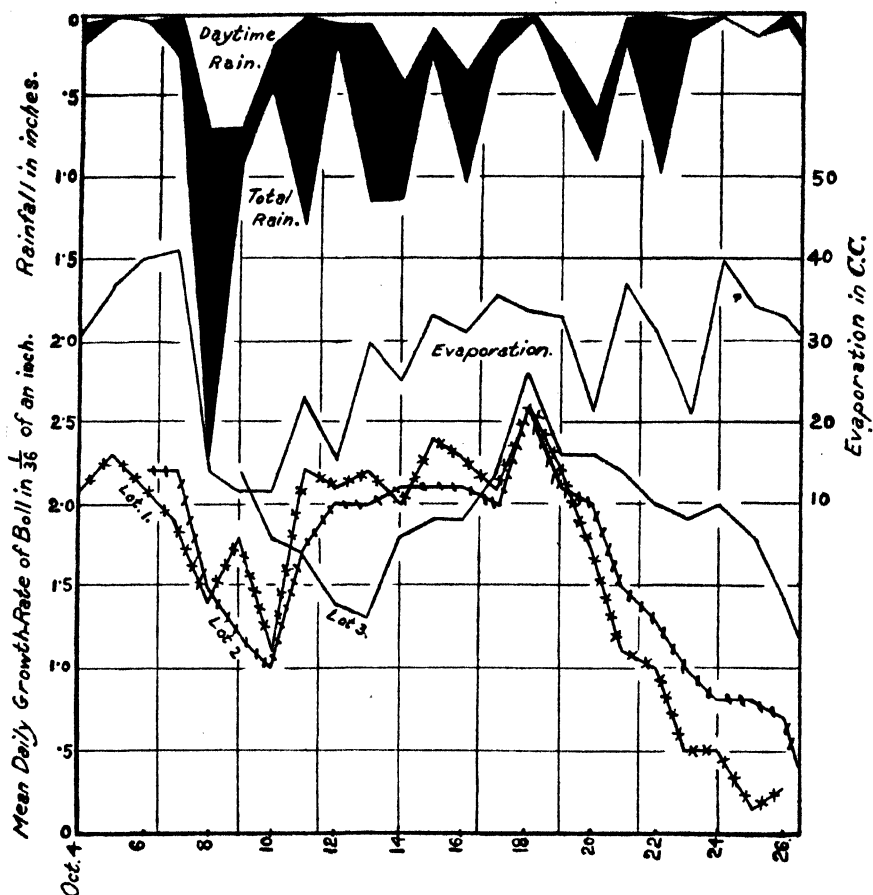


FIG. 13. Growth-rate of boll, evaporation, and rainfall.

THE GROWTH-RATE OF THE BOLL AT DIFFERENT STAGES DURING THE FLOWERING PERIOD.

The results which are reproduced graphically in Fig. 14 were obtained by tagging sixty flowers (on the second group of plants) weekly throughout the flowering period and measuring daily the growth-rate made by each lot for the six days following the flowering stage. Bolls which were shed within this period are not included in the results; their number is shown on the graph within the circles. The coefficients of variability of the daily growth-rates and also the daily rate of boll-shedding for the whole group have also been included.

The continued decline in the growth-rate of lots 5, 6, 7, and 11 is the first point deserving comment. It will be seen that this decline was accompanied by a marked increase in the coefficients of variability of the

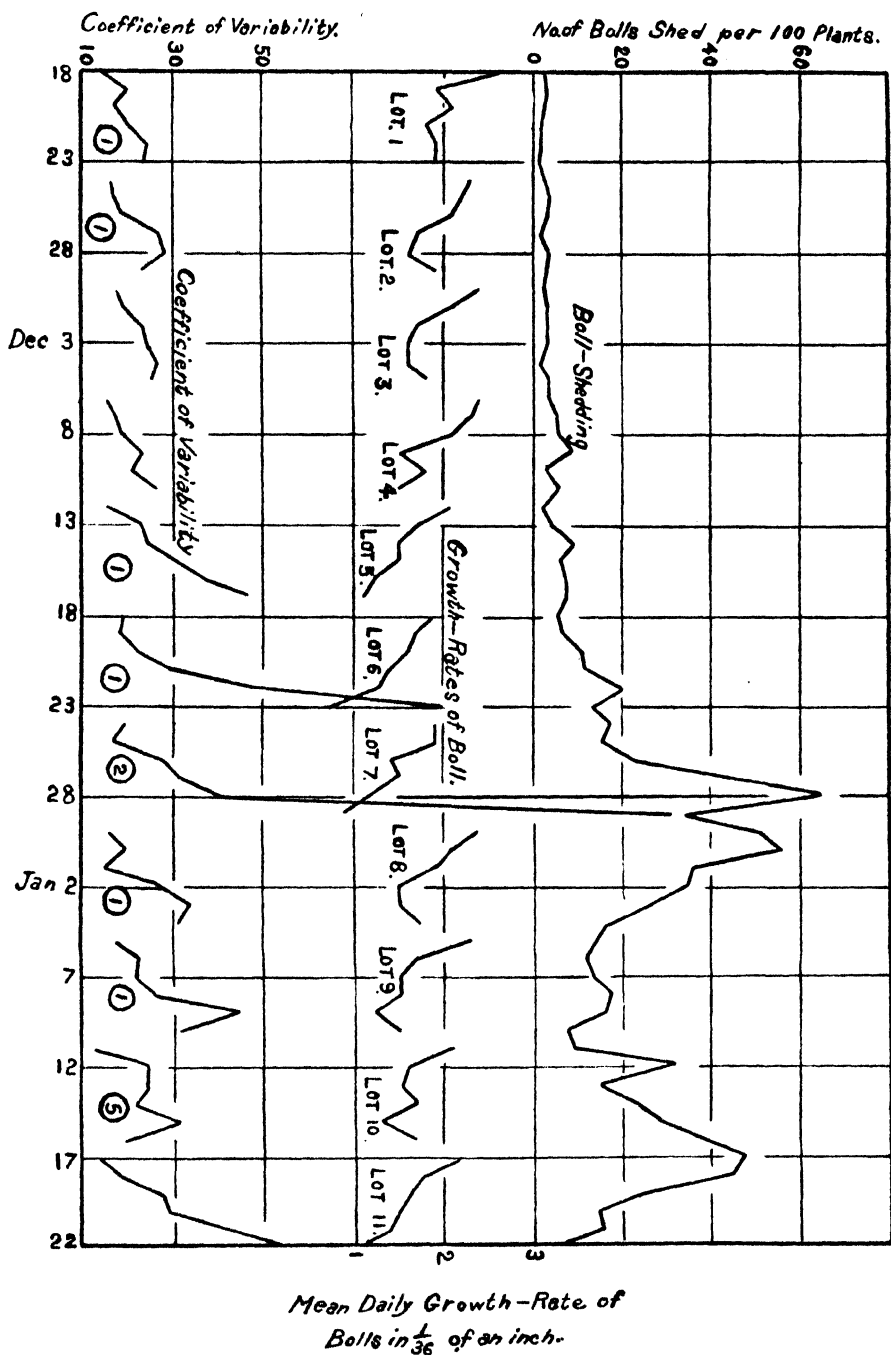


FIG. 14. Daily growth-rates of boll for six days. Coefficients of variability of daily growth-rates and daily rate of shedding for Gp. II.

growth-rates, and that it became especially pronounced during the periods of heavy shedding. It seems evident that this continued retardation is due to the fact that the growth of a relatively large proportion of bolls at this period was subnormal, that many failed to undertake the normal recovery due to the stimulus of fertilization and were ultimately shed. It is interesting to observe that, even when little or no shedding occurred, the coefficients of variability tended to increase for a few days following anthesis, and to diminish as recovery took place. It may be seen that the amount of growth which was made on the first and second day after the open flower stage diminished rather steadily up to December 25, and then increased. It is not improbable that this was due to a progressive stringency in the supply of assimilates available for fruit development, and that the recovery which subsequently occurred was due to the augmented food supply, which presumably occurs after a wave of shedding and tends no doubt to diminish the liability of the boll to undergo abscission.

GENERAL CONCLUSIONS AND DISCUSSION.

The results obtained disclose the intimate connexion which exists between growth and abscission. It was shown in the first place that the tendency of the plant to shed its young fruit was relatively small until growth in the main axis had almost ceased.

Both of these phenomena, the cessation of growth in the main axis and the more pronounced susceptibility to shedding, were attributed to a correlation factor, which was introduced as a result of the development of fruit on the basal fruiting branches. It seemed probable that this factor was in some way associated with a deflexion of assimilates from the apical to the basal part of the plant, and that consequently the greater liability of young bolls, produced during the latter portion of the flowering period, to undergo abscission resulted from their inability to obtain the assimilates necessary for development in the presence of a large number of more mature bolls. An important consequence of this hypothesis, in support of which an experiment by Ewing was cited, is that the elimination of fruit by abscission or in other ways diminished the susceptibility to shedding; this is due, no doubt, to the fact that a reduction in the number of competing centres necessarily augments the supply of elaborated food. It should also follow as a result of the progressive stringency in the supply of assimilates that a certain amount of shedding would occur, even under constantly favourable environmental conditions. In a word, it was concluded that the amount of shedding which takes place during any given period is determined on the one hand by the rate at which food is elaborated by the plant and on the other by the rate at which it is withdrawn by the developing fruit, and that consequently the advent of any factor which tends to limit elaboration augments the rate of shedding.

The next phase of the inquiry dealt with the effect of certain environmental factors upon the daily growth-rate of the main axis and the rates of shedding. It was found that dark, humid days, during which the rate of evaporation remained low and on which a great deal of rain generally fell, resulted in marked retardation in the growth-rate of the main axis, and that in the later stages of the plants' development they were the invariable precursors of augmented rates of boll-shedding. It was assumed that the occurrence of external conditions of this nature must operate to check the rate at which food was elaborated, and that this check was the cause both of the retardation in the growth-rate of the main axis and of the augmented rates of shedding. The absence of laboratory facilities rendered it impossible to place this hypothesis on an experimental basis. It was shown, however, that the removal of all the foliage leaves was followed within from three to eight days by the dropping of almost all the young fruit. It was also found that the removal of all except a few fruits considerably prolonged the interval elapsing between the application of the stimulus and the completion of abscission. Moreover, it was found that pruning off the fruit, and thereby diminishing the number of competing centres, resulted in augmented growth-rates of the main axis.

Inasmuch as previous work has consistently pointed to a water-deficit in the body of the plant as being the most constant cause of shedding, an experiment was made to determine whether incipient drying was sufficiently pronounced, under the humid conditions of St. Vincent, as to markedly retard the growth of the plant. As the result of measurements which were made both in the morning and the evening, it was found that growth was only slightly inhibited while the sun was up. This was not unexpected, for the desiccating power of the aerial environment is normally very small during the period in which cotton is cultivated in St. Vincent. In view of this, and the considerations just advanced, the conclusion seems inevitable that the growth-inhibition which precedes shedding in St. Vincent is not the result of pronounced incipient drying, but is due rather to a retardation in the rate at which assimilates are produced by the plant. It should be emphasized, however, that a series of abnormal saturation deficits would also check the rate at which carbohydrates are elaborated.

Finally, it was shown that the growth-rate of the boll fell normally into two distinct stages. During the first period growth continued for some four to five days after anthesis, but at a declining rate. Growth throughout this period was apparently in no way dependent on fertilization. During the second stage the rate increased until finally it declined. The initiation of this stage was found to be dependent on fertilization. It was concluded that the stimulus supplied by fertilization operated in some way to ensure a movement of assimilates into the boll. In the absence of fertilization the boll underwent a steady decrement in growth until it was

finally shed. That the destruction of pollen by rain was responsible for a small proportion of the shedding there would seem to be no doubt, but the more generally constant cause, it was concluded, was due to an interruption in the supply of assimilates entering the boll during the critical stage of growth-retardation which followed anthesis. The occurrence of a check in the growth-rate at this period seemingly prevents a variable proportion of the bolls from undertaking the augmented growth of the second stage, in spite of fertilization. Inasmuch as the factors responsible for the movement of elaborated food in the plant are still quite unknown, it is useless to speculate as to why one boll is able to survive while a neighbouring one undergoes abscission. The phenomena of physiological correlation are presumably in some way responsible.

Before concluding, it is important to note that negative growth-rates are normally shown for some one to three days prior to the completion of abscission. It would seem that the failure to secure the normal supply of assimilates must diminish the water-absorbing power (7) of the boll until a stage is reached at which the tension in the water columns of the plant leads to a suction of water from the boll. It may well be that this is not only the cause of the negative growth-rates, but that it is actually the factor which initiates abscission. It will be evident that any factor which injures the boll,—insect punctures, fungous diseases, &c.—will bring about abscission, provided the injury is sufficiently pronounced as to interrupt the movements of elaborated food into the boll.

SUMMARY.

1. A survey was made of the external and internal factors affecting the shedding of bolls and flower-buds in St. Vincent.

2. The susceptibility to shedding is relatively small in the earlier stages of the flowering period, but becomes much more marked in the later stages. It was found that the susceptibility becomes especially pronounced after the occurrence of growth-cessation in the main axis.

3. Both the cessation of growth in the main axis and the augmented susceptibility to shedding were attributed to a correlation factor which tended to deflect the supply of elaborated food from the apical part of the plant to the fruit developing on the basal fruiting branches.

4. The growth rate of the main axis was retarded on overcast, humid days. Periods of daytime rain, low rates of evaporation, and little direct solar radiation were also the precursors of augmented rates of shedding.

5. The retardation in the growth-rate of the main axis and the augmented rates of shedding were both attributed to a check in the assimilative activity of the leaves.

6. Removal of the foliage leaves from plants on which there were a number of maturing bolls resulted in the abscission of 96.5 per cent. of the young bolls within a period of nine days. The interval between the operation and the maximum rate of shedding was approximately similar to that normally occurring between the causative environmental conditions (cf. 4) and the completion of abscission.

7. The growth-rate of the main axis was only slightly retarded during the hours of sunshine, from which it was concluded that incipient drying was not pronounced under the humid conditions of St. Vincent.

8. The growth-rate of the boll declined for some days following anthesis; recovery was found to be dependent on fertilization.

9. It was concluded that the metabolic transformations which preceded the formation of the absciss layer were especially liable to be initiated during this period of growth-retardation.

10. Negative growth-rates were generally shown by the boll for some one to three days prior to the completion of abscission. It was suggested that inability to secure the assimilates necessary for normal development diminished the water-absorbing power of the boll until a stage was reached at which the tension in the water-columns of the plant led to a suction of water from the boll. This, it seemed, was the cause not only of the negative growth-rates, but was actually the factor initiating abscission.

11. The general conclusion was drawn that the proportion of shedding over any given period was the resultant of two opposing factors, the rate at which food was synthesized by the plant and the rate at which it was utilized in the maturation of the fruit; and that any check in the former augmented the rate of shedding.

12. It was emphasized that any factor which injured the boll—fungous and bacterial diseases, insect depredations, &c.—caused the shedding of the boll, provided the injury was sufficiently pronounced as to interrupt the translocation of food into the boll.

In conclusion, the writer wishes to record his indebtedness to Mr. C. A. M. Marshall for his indefatigable assistance throughout the progress of the work.

ST. VINCENT,
March 1922.

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Growth Studies.

III. A 'Volumometer' Method of measuring the Growth of Roots.

BY

J. H. PRIESTLEY AND W. H. PEARSALL.

With one Figure in the Text.

ONE of the outstanding difficulties of studying growth in plants is the making of continuous measurements upon a single plant grown under controlled external conditions. In studies of animal growth this difficulty does not arise, since it is a relatively easy matter to weigh the animal at stated intervals. In plants, however, measurements of total weight are of doubtful value, owing to the large percentage of water present (about 90 per cent. usually), which may vary without reference to any actual change in protoplasmic mass. It is, moreover, difficult to weigh fresh plants accurately without such precautions as must destroy the plant as a growing individual, and of course dry weight determinations involve the entire destruction of the individual. In addition, even length measurements become practically impossible when dealing with branched structures such as older roots, and their value must always remain slight, on account of variations in the thickness and structure of the organs at different stages of development.

It was an attempt to overcome some, at least, of these difficulties that led the authors to devise an apparatus for measuring the volume of roots at different stages of growth. The essential idea involved in this apparatus was that roots should remain in a fixed position in some container which would allow of their being grown under suitable conditions for a comparatively lengthy period. The container had, therefore, to provide for the accurate measurement of small volumes, and yet had also to be of considerable size to permit extensive root development. In addition, it had to be provided with a means of renewing the nutrient solution.

In the following paragraphs an account is given of an apparatus devised to meet these conditions and used with satisfactory results.

The container (D C in figure) consisted of a glass vessel holding about 300 c.c. of water, with a narrow neck of about 2.5 cm. diameter. The neck was provided with a blue porcelain point, directed downwards. The

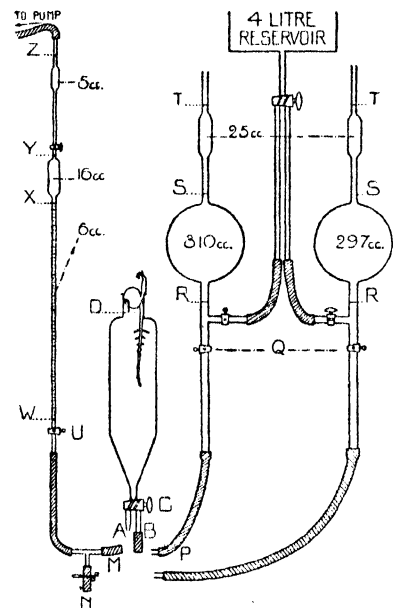
tapering base of the container terminated in a three-way tap, the bore of the passages being 2 mm.

Large seeds, such as beans, were used, and the germinated seed was wedged into the container neck, care being taken that the root was on the side of the neck opposite to the porcelain point, and the latter clearly visible. The container was filled with solution by allowing the latter to enter through the basal tap until it just touched the porcelain point. The volume of solution entering was measured, and this obviously would grow less as the

roots increased in size—the decrease in volume of solution representing the increase in the volume of the roots.

A number of containers could be used with the same refilling and measuring apparatus. Generally six were employed, each containing a plant under observation. Except during a measurement, the containers were kept in a large water bath at a constant temperature of $15^{\circ}\text{C.} (\pm 1^{\circ})$. The bath was covered over so that the roots were kept in the dark, the shoots being in the light.

The containers were filled from a series of bulbs (R, S, T, &c., see figure) fed from a four-litre reservoir of nutrient solution. As the individual containers held various amounts from 315 to 358 c.c., the bulbs were arranged so as to run in any one of the four values 297, 310, 322, and 335 c.c. with the minimum



Apparatus for measuring growth of roots in volume; for explanation see text.

number of readings. With the arrangements figured, the discharge of any one of these volumes required only two readings of meniscus levels (at T or S and at R). The diameters of the tubes on which the level marks were made was about 5 mm., so that the readings involved only a small error at most. The point R was kept at a uniformly higher level than the top of the container.

The containers could thus be nearly filled by running in a known volume from these bulbs. The final small amount of solution was added from a measuring tube, WZ, which consisted of two bulbs, and below them a long glass tube graduated in 0.01 c.c. Any volume between 0 and 6 c.c. or 16 and 27 c.c. could be run in from this tube, and in conjunction with the various filling bulbs described it was thus possible to fill and measure the volume of solution in any container between 298 and 363 c.c. The

internal diameter of the tubes Z W was less than 2 mm. A tap at N allowed for the removal of air-bubbles.

The further details of the apparatus can best be realized from a description of its use in measurement. A container holding a plant whose roots were to be measured, and filled with the nutrient solution supplied to it on the last occasion that the volume was measured, was clamped *vertically* in a stand. The connexion M, at the base of the measuring tube, was attached to the outlet A of the container. A pump connexion was then attached at Z, and the solution from the container sucked up the measuring tube as far as was necessary. The tap U was then closed and the pump disconnected. By adjusting U, the level of the fluid in the tube was then allowed to fall to Z, Y, or X as required. Tap C was then turned and the solution in the container allowed to run off through B, until the meniscus in the container was just level with the top of the tap outlet at C. Before making the final adjustment here, A was opened to allow the pressure in U M to become equal to that of the atmosphere, since otherwise it would vary with the height of the column in the measuring tube, and a volume error would be introduced. When C was adjusted the connexion on B was attached to P, no air being included. C was then opened again and the required volume of fresh nutrient solution run in from the bulbs, by adjusting tap Q. B was then closed and A opened, when, by adjusting tap U, the final small amount of fluid was run in until the surface of liquid in the container just touched the porcelain point in the neck. The reading on scale W X then gave the volume added from the measuring tube. As the roots of the plant grow, this becomes less, and the decrease in this reading indicates the volume increase in the roots. If there is any doubt as to the accuracy with which this end-point has been measured, the solution can be sucked up into the measuring tube and the final operation repeated.

The sources of error in this apparatus are obviously due chiefly to the number of readings of meniscus level. In any series of observations with the same container, however, these readings are made at the same six places in the apparatus, so that this source of error is continuous throughout the readings. Since all of these levels, except that at D, are observed in fixed and narrow tubes, they should permit of accurate determination. In the mouth of this container, however, the accuracy of the end-point in filling must depend largely on whether the container is fixed in an exactly vertical position or not. In our containers, the body was cylindrical, and its correct position was ensured by getting the edge in exact alinement with the upright of a stand behind it.

Air bubbles in the apparatus are another source of error, but they are easily seen and as easily avoided. For obvious reasons, the temperature of the apparatus should be kept approximately constant at the different readings.

As the whole apparatus is in practice filled with liquid from a large reservoir at room temperature, the error introduced by temperature changes during the course of the reading is certainly well below other sources of experimental error.

Experience shows that if the plant used is only wedged into position in the neck of the container by means of its cotyledons, it is liable to move as the cotyledons decrease in size. In future, therefore, it would be advisable to fix the plant upon a ledge in the neck of the apparatus.

The accuracy of the results obtained with this apparatus can be estimated from the first series of readings made with it. Five containers were in use at the time, each containing a growing plant. After each reading, the container was emptied and the whole reading repeated. The average divergence of the second reading from the first was 5.50 ($\times 0.01$ c.c.), the figures (unit = 0.01 c.c.) for the individual containers being as follows:

No. of container	1	2	3	4	5	
No. of observations	26	28	26	24	26	Total 130
Average divergence	5.58	5.64	5.39	5.00	5.89	Mean 5.50
Maximum divergence	12	15	19	12	19	

The largest divergence is 19 ($\times 0.01$ c.c.), and the usual character of the volume changes recorded (readings being taken every second day) can be gathered from the following summary of the distribution of the actual volume changes obtained within a few arbitrarily chosen limits:

Increase in volume 0.01 c.c. per 2 days	Over 100	100-60	60-20	20-10	below 10
No. of observations	5	9	69	26	21

This set of observations lasted considerably over a month and included not only readings when growth was practically complete, but also the temporary cessation of growth following on the development of lateral roots. During the normal development of bean roots, volume changes of the order of 0.2 to 0.6 c.c. (per two days) are to be expected, after the appearance of secondary roots. The earlier stages of root growth are probably better observed by direct measurements of length as in Leitch's experiments (1), or by the method of Neilson-Jones (2).

One further point justifies our assumption that the volume changes observed lie outside the normal range of experimental error. The main results obtained are reproducible, the growth curves of individual plants being of the same general type. We hope to present conclusions based on these results in a subsequent paper.

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On the Organization of Growth and Differentiation in the Stem of the Sunflower.

BY

D. THODAY, M.A.,

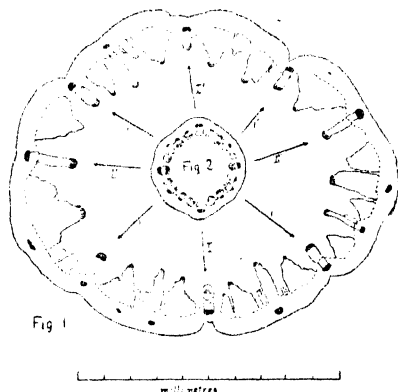
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With Plate XVII and ten Figures in the Text.

THIS paper is the outcome of an inquiry into the meaning of the irregularity of the zone of secondary wood in the old stem of *Helianthus annuus*, which in this respect stands in marked contrast to the stems of most woody plants.¹

The stem of a young Sunflower plant has the usual ring of vascular bundles, bulging a little in conformity with the prominence of the leaf-bases, but otherwise regular (Text-fig. 2). The medullary rays are not very wide, so that the arrangement of the tissues is not conspicuously different from that in young twigs of a multifasciculate type of structure. It is during growth in thickness that the contrast develops.

In the secondary growth of most woody twigs, secondary tissue is added more or less regularly and uniformly by radial activity of the cambium. The primary xylem and the pith appear to remain unaffected by the mechanical disturbances that strain the outer tissues.



TEXT-FIG. 1. Transverse section through the middle of the third internode of a stem over a centimetre in diameter, showing the irregular formation of secondary wood. *I, I'*, median and *i, i', ii, ii'*, lateral trace bundles of leaves at the node above; *II, II'*, median trace bundles at the next (fourth) node. Fibres are shown black.

TEXT-FIG. 2. (Inset in Text-fig. 1 on same scale.) Same region of a young stem.

¹ Compare Thoday, Botany, Cambridge Univ. Press, 1919, Fig. 35, p. 138.

In the Sunflower stem, on the contrary, the pith increases greatly in diameter and the bundles become widely separated. Text-figs. 1 and 2 represent sections through the third internode above the cotyledons of two plants of different age. Both are drawn to the same scale and the inset of Text-fig. 2 in Text-fig. 1 serves to emphasize the point in question. The diameter of the pith has increased approximately threefold during the growth that has occurred between the two stages represented. Both plants were grown in the same plot, and at the earlier stage the diameters of their stems were of the same order of magnitude.

In the older stage certain of the primary bundles are still readily distinguishable. Six of them (*I, i, i* and *I', i', i'*), which belonged to the pair of leaves at the node next above, are conspicuous by reason of their small radial extent—the fascicular cambium has not been very active. Two other bundles (*II* and *II'*) are the median bundles belonging to the pair of leaves at the second node above. In these the cambium has been more active radially. But all these eight bundles and a few others are alike in their small width, which is little if at all greater than the width of the original primary bundle (Text-fig. 2). The width of the intervals between them, on the other hand, has increased enormously, and in these intervals broad wedges of secondary wood have been formed.

In the enlarged pith, lines of strain are expressed in the arrangement, shape, and dimensions of the cells (Text-fig. 3), which clearly indicate that the pith has not itself been the active agent in the expansion, but has suffered enlargement more or less passively. Indeed, in still older stems it yields under the strain and becomes hollow (compare Plate XVII, Fig. 5). The active growth has been located in the cambial region and the expansive force exerted in the tangential direction.¹ In this tangential growth the cambium of the principal bundles has taken little or no part.

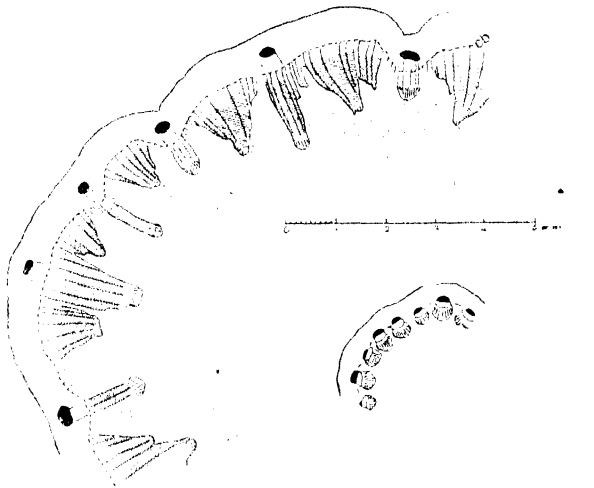
Further information is obtained from a closer study of the older section. Text-fig. 3 illustrates a part of it on a larger scale. The radial lines in the wood mark the direction of rows of elements formed by individual cambium cells: their divergence in parts of the sectors between the principal bundles is clear evidence of tangential growth at these points (see also Plate XVII, Fig. 4). It is also evident, however, that the sectors have been augmented by the extension of cambial activity into the adjoining medullary rays, which have widened considerably to accommodate it. On either side of each of the principal bundles the rays have widened still further, and are now bridged by cambium. Here the cambium has not given rise to any secondary wood, and therefore is either recent or relatively

¹ Cf. Sachs, Textbook, p. 125: 'This increase in diameter of stems which accompanies, or even for a short time outlasts, the growth in length, is frequently occasioned mainly by the tangential extension of the outer layers of tissue, while that of the pith does not keep pace with it. The pith will then split and the stem become hollow.' But Sachs appears to refer here rather to the cortical parenchyma.

inactive. Evidences of strain in the inner part of the rays show that it is in the cambial region that the tangential growth has occurred.

Accommodation phenomena. A full understanding of the unequal distribution of tangential growth involves a knowledge of the course of the bundles and secondary wood-sectors in adjacent parts of the stem, for accommodation is of course mechanically necessary. The widening of the rays flanking the principal bundles is probably in part an accommodation phenomenon of this kind, correlated with the growth of wood-sectors above the outgrowing leaf-trace bundles.

Other examples are found at the base of the stem. For instance, the median paired bundles of the cotyledonary traces become widely separated



TEXT-FIG. 3. Parts of Text-figs. 1 and 2 enlarged. The 'radial' lines in the wood show the direction of rows of elements formed by cambial cells. The broken lines in the pith represent lines of strain. Secondary wood shaded; rows of vessels indicated in primary xylem; fibres black; *cb*, cambium.

in conformity with the tangential growth of a massive wood-sector in the internode above. At a later stage the interval is bridged by secondary wood.

Another example is afforded by the diminution of tangential growth downwards in the lowest epicotylar internode and the hypocotyl. As the root is approached the pith suffers less and less enlargement and tapers to a point in the transition region. This is a natural accommodation to the absence of pith in the root. There is a complementary increase in radial activity of the cambium down to the root, where tangential growth would obviously be unsuitable to the mechanical requirements. It is largely for this reason that the base of the stem is so much more woody, compared with parts a little higher, than mere difference of age would lead one to

expect.¹ There are, however, other correlations between hypocotyl and root which require further investigation.

Course of bundles. As the arrangement of the leaves is at first opposite and decussate, but later alternate, the course of the leaf-trace bundles shows a corresponding change. In a plant growing under favourable conditions the change of phyllotaxy begins after about four pairs of opposite leaves have appeared. The trace bundles of the third pair pass down through three internodes and then usually fork, but sometimes deviate without forking, so avoiding the incoming leaf-traces of the first pair. The median bundles of the first pair similarly fork past the lateral cotyledonary trace bundles. If the leaves at alternate nodes were exactly superposed, the bundles would typically pass through two internodes, then fork, each branch being joined by a similar bundle from the node above. This ideal arrangement is only approximated to even at the base of a vigorous plant.² In a particular stem, in which the bundles were traced in detail, the fourth pair of leaves already showed a small displacement, the next two leaves deviated still more and were separated by a short internode. The lateral displacement of the leaves involves the leaf-traces also and the bundles tend more and more to miss incoming leaf-trace bundles at lower nodes and so to traverse a larger number of internodes independently.

The transition to an approximately two-fifths phyllotaxy is rapid. When this arrangement is fully established the bundles can be followed for about five internodes, sometimes even farther, before they are joined by one or more minor bundles, after which they traverse one or two more internodes, sometimes fork to avoid incoming bundles, and, diminishing in size, finally attach themselves to neighbouring bundles. Sometimes they end blindly as primary bundles; but secondary continuations link them to neighbouring strands.

There is nothing stereotyped about this disposition of the bundles. The facts can best be understood as a solution of the problem of accommodating the traces of an increasingly rapid succession of leaves. The space available diminishes downwards: firstly, by reason of the entrance of other traces at each node; secondly, because towards the base of the plant all the primary tissues are on a smaller scale. Trace bundles from the upper leaves are therefore crowded into narrower spaces and ultimately unite with one another. It is these synthetic traces that grow tangentially and that form the massive wood-sectors so conspicuous at the base of the stem. They always communicate directly with the upper, still expanding part of the shoot. Moreover, their growth is correlated with that of the

¹ It may be thought that these accommodations have been emphasized unduly, but Jeffrey and others who have started with a phylogenetic point of view seem to have entirely missed them. See below, pp. 503-5, for further discussion.

² But a close approximation to it is found in starved dwarf plants, where it persists through several internodes. Cf. p. 502.

Figs. 5 and 6, are photographs of the larger section; they show the hollowness of the pith and the lines of strain in the outer still intact part of it.

Incidentally the structure of the upper part of the stem is the better adapted to tangential growth, since the independence of the leaf-trace bundles through greater distances means a corresponding continuity of medullary rays and less frequent cross-connexions between the tangentially growing sectors. Frequent anastomosis would obviously hinder the tangential growth of the anastomosing strands, for the fibrous cross-connexions would offer greater resistance to the separation of the strands than the yielding ray parenchyma. Radial segmentation of the woody cylinder is indeed a *sine qua non*. Active tangential growth in the cambial region outside a continuous zone of wood is hardly conceivable.

Anastomosis of phloem strands at the node and elsewhere. The independence of the leaf-trace bundles for a long distance entails no serious disadvantage in relation to the conduction of water, since the supply comes from below and serves the one leaf to which it is directly transmitted. For the translocation of food, however, which comes from the leaf and is required mainly above, the path would be greatly lengthened. It is therefore interesting to find that a short circuit is established at the node, by lateral union of phloem associated with the incoming leaf-trace and the phloem of adjoining minor bundles.

The details require further elucidation in the petiole on the lines of the present study of the stem, but the following brief outline, together with Text-fig. 5, will suffice in the present connexion. In the petiole of a large leaf there are a number of small bundles as well as the three principal ones. The former appear to anastomose in rather intricate fashion and one or two larger ones unite with the two lateral bundles. In the base the remainder cluster round the principal bundles as these diverge. Many of the smallest consist, even in the case of a mature leaf, of phloem only, and in the others the xylem dies out in the nodal region. Thus, just above the level at which the trace bundle enters the vascular zone of the stem, there are associated with it a number of small strands of phloem, which encircle the xylem (Text-fig. 5 (6)).

At the same level cambium is continuous across the rays. In the case figured the two small bundles are the branches of a small bundle which has forked just above. At the approach of the leaf-trace bundle these branches diverge, the cambium ring bulges inwards and divides to admit the xylem of the incoming bundle. Meanwhile the phloem strands that encircle the latter take their place as they arrive, along with a few other phloem strands already present, outside the cambium, and follow it when it divides. The free ends of the divided ring then gradually withdraw into the undistorted parts adjoining, and the associated phloem strands crowd together, along



TEXT-FIG. 5. (1) to (8) Sect ons of a median leaf-trace bundle at right angles to its course from the leaf-base through the node, showing accompanying phloem strands, which form a plexus on each side in the node, connecting with the phloem of adjoining bundles. (9) A lateral trace bundle entering at the node showing similar features. Lignified fibres heavily shaded; phloem finely shaded; secondary wood cross-hatched; rows of vessels indicated in the primary xylem. Xylem of small satellites of the trace bundle black. (Resin canals omitted from (5) and (7).)

with other incoming strands on both sides of the incoming bundle. Thus a phloem plexus is formed on each side, in close association with the phloem of the adjoining bundles which communicate with the upper expanding part of the shoot and later form part of the secondary synthetic strands.¹

It is not only at the node, however, that communication is established laterally between the principal phloem strands. Anastomosing strands are frequent between all the bundles, in the internodes as well as the nodes, so that the phloem in its lateral continuity stands in marked contrast to the xylem.

Ontogeny of the leaf-trace bundle. As already observed, the leaf-trace bundles in the upper part of their course stand out in sharp contrast with the synthetic strands, not merely by reason of their isolation by flanking parenchyma, but also because the fascicular cambium behaves differently. Not only is it relatively inactive, but, as Jeffrey and his collaborators have observed in this and other herbaceous stems, the secondary xylem that it forms is for a time largely, if not wholly, parenchymatous.

Before considering the possible significance of these peculiarities, it is necessary to complete the picture of the ontogeny of the stem by adding to the account of the later stages already given a description of the earlier stages, with special reference to the ontogeny of the individual leaf-trace bundle.

The primary xylem is characterized by rows of vessels, mostly spiral, separated by bands of parenchyma two or more cells in width, and often diverging in a fan-like manner towards the pith. The typical secondary wood contrasts sharply with the primary, and consists of fibres and large pitted vessels, with medullary rays. But, notwithstanding the histological contrast, the rows of elements can often be traced continuously right through the primary and secondary xylem into the cambial zone. This continuity is only obscured where in the secondary wood the growth of large vessels has led to distortion, or where in the protoxylem the oldest vessels have collapsed and adjacent cells have encroached upon them. It is often clearest in the upper parts of the bundle, where the secondary xylem is at first largely parenchymatous and not distorted by vessels (Plate XVII, Fig. 1).

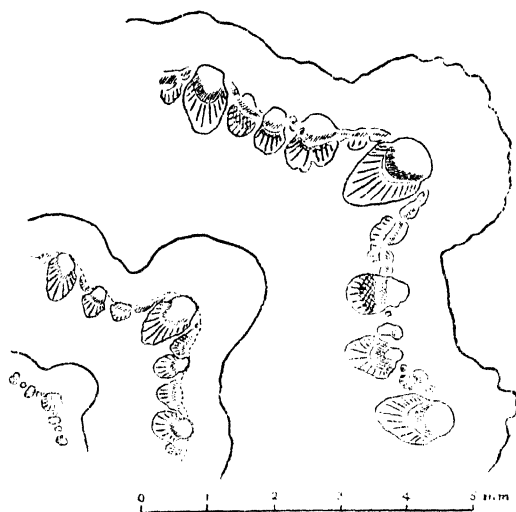
Examination of the early stages of development of the bundles confirms the inference that the primary and secondary xylem are formed by the activity of the same cambium and are only distinguishable histologically.

If a vigorous plant is selected while it still shows no sign of the inflorescence, but is already producing a succession of large leaves, trans-

¹ In the case figured the axillary growing-point was latent and no vascular supply to it was even foreshadowed. Where an axillary bud is developed its vascular cylinder opens inwards and joins the vascular zone of the stem above the median leaf-trace bundle.

verse sections at short distances from the apex may legitimately be taken to represent, approximately, different stages of the ontogeny at one level in that region, for the size of the mature primary bundles of successive leaf-traces has become fairly constant. Text-fig. 6 represents corresponding portions of three such sections, including the principal leaf-trace, drawn to the same scale. It exhibits in a striking manner the great increase in size of the bundles, their progressive separation with the growth of the whole stem, and the interpolation of additional bundles in the widening intervals. In the growth of the individual bundles it is to be observed that the rows of vessels increase not only in length but in number.

Still nearer the apex the bundles are represented by small groups of procambial cells separated by narrow radial bands of developing parenchyma.



TEXT-FIG. 6. Corresponding portions of three sections, each including the three bundles of a leaf-trace, at different levels near the apex of a vigorous plant not yet beginning to flower. Phloem and procambium shaded except in earliest stage.

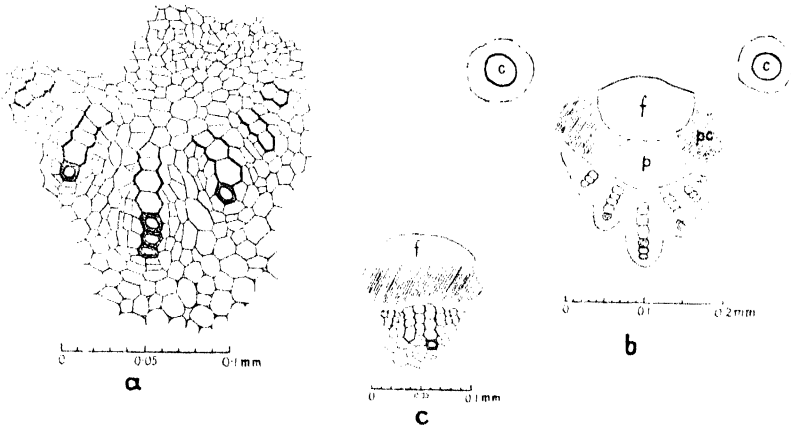
These groups increase in size and soon become distinguishable into an outer part, with the cell contents rather less dense, which ultimately forms the fibres, and an inner crescent of denser procambium.

The differentiation of this procambium does not correspond to the current view of the development of a collateral vascular bundle, according to which the procambial cells begin to differentiate on the outside as protophloem and on the inside as protoxylem. In this stem, cambial activity begins very early on the inner margin of the procambial crescent, and the whole of the xylem is the product of this. Moreover, the procambial crescent grows laterally, the cambium extending also along its inner margin.

In the earliest stage of the differentiation of vessels clearly recognizable

in transverse section two cells are distinguishable by their larger size and less dense contents, one lying outside the other near the inner margin of the procambial crescent and in the median line. By the time the number of cells in the row has reached about four, the innermost has been differentiated as a vessel, and two other similar rows, each of two or three cells, are distinguishable on either side of the first row a little removed from it. Text-fig. 7, *a*, represents a slightly later stage. The first vessel has been differentiated in each of the lateral rows and the third in the median row, where the two oldest already show signs of weakening. Laterally, still other rows have been initiated.

Between the rows of vessels the cells cut off by tangential divisions



TEXT-FIG. 7. *a*. Nylem and parts of adjoining tissues of a young bundle near the apex of the same plant as Text-fig. 6, showing 'cambial' origin of protoxylem. *b*. Same bundle on a smaller scale: *f*, fibre initials; *p*, differentiating phloem; *pc*, procambium; *c*, canals associated with the bundle. *c*. Young median trace bundle just above the cotyledons, belonging to one of the leaves at the second epicotylar node of a young plant. Here also the protoxylem is 'cambial' in origin.

divide further by radial walls, particularly those in immediate proximity to the vessels, and thus form round the rows close sheaths of small cells with dense contents. Midway between the rows the cells divide less and their contents become less dense. In a mature bundle the sheaths are collenchymatous and conspicuously chlorophyllous,¹ without air-spaces. For variable distances from the pith they are separated by narrow bands of larger cells with air-spaces but less chlorophyll.

At the stage corresponding to Text-fig. 7, *a*, the differentiation of phloem was already proceeding in the median part of the procambial crescent, but the ends of the crescent were still in the procambial condition and actively growing (Text-fig. 7, *b*). The crescent extends in this way at both ends and the differentiation of phloem follows in both directions from

¹ Indeed, the vascular zone is the greenest part of the stem!

the centre. Cambial activity also extends along the inner margin and additional rows of vessels appear.

At a later stage the cambium often adds also to the phloem, but it is difficult to tell how much of the phloem of a mature bundle has originated from it.

The characteristic fan-like divergence of the rows of vessels towards the pith is the resultant of several factors, and varies in degree. The cambium is more or less curved *ab initio*, and the rows of elements tend to be at right angles to it. The curvature becomes more obvious as the cambium, following the procambial crescent, extends laterally. Later, the expansion of the fibres and the formation of additional phloem by the cambium diminish its curvature. On the other hand, the cells between the rows of vessels continue to grow and so widen the spaces between them and maintain the divergence. At the same time the surrounding parenchyma is growing, and mutual adjustment between this and the bundle, which bulges into the pith within and into the cortex without, is obviously necessary. The divergence is most pronounced where the bundles reach a large size and the general primary expansion is most vigorous. Towards the base of the stem, where the bundles are small and growth generally less vigorous, the rows of vessels are often nearly parallel (cf. Plate XVII, Fig. 1).

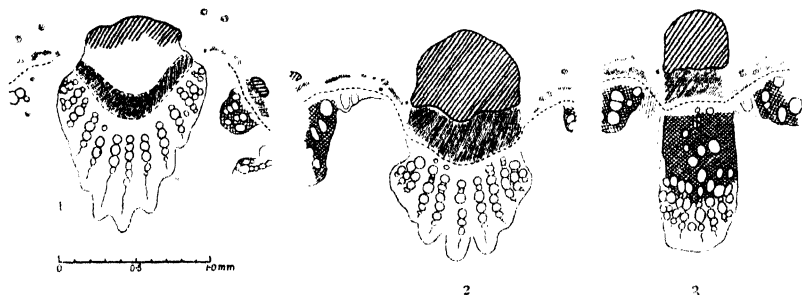
The leaf-trace bundle at different levels. At a given level the bundles that belong to the next node are the first to appear and are precocious in their development. The trace bundles of younger leaves are successively interpolated and their development shows a corresponding lag. When the primary growth comes to an end the trace bundles of two or three leaves are already full grown. Further growth in the others is secondary in character.

The structure of different regions of an individual trace bundle of a full-grown leaf varies accordingly. For two or three internodes (in the upper part of the stem) the xylem is more or less constant in form and size and consists entirely of primary tissue. Most of the vessels are spiral or annular, or partly annular and partly spiral. The last one or two in each row show reticulations or include an occasional pitted vessel. Farther down the amount of primary xylem diminishes and secondary xylem is associated with it in increasing amount (Text-fig. 8). It may be inferred that in the first two or three internodes the trace bundle reaches practically its full size while growth in length is still proceeding and the surrounding parenchyma is still growing actively; and further that in the uppermost internode it is not only precocious but reaches its full size before the primary growth of the rest of the tissues is complete.

So long as growth in length continues, vessels put out of action by elongation are doubtless replaced, and the last vessels are such as could be

formed only when growth in length comes to an end. But even when these are pitted vessels they are smaller in diameter than the spiral vessels formed during the period of most active growth of the bundle, and far smaller than the pitted vessels typical of the secondary wood (see Text-figs. 8 and 9). The bundle may therefore be said to have a grand period of transverse growth which corresponds to that of the leaf to which it belongs but is more or less independent of that of the internode in which it occurs.

At the end of this period the cambium becomes sluggish and remains so for a considerable time. In the large bundles of the upper part of the stem the cambial zone often develops air-spaces and becomes parenchymatous, especially the median part, so that a definitive cambium cannot be located. This applies throughout the upper part of the bundle where its development is completed during the primary phase.

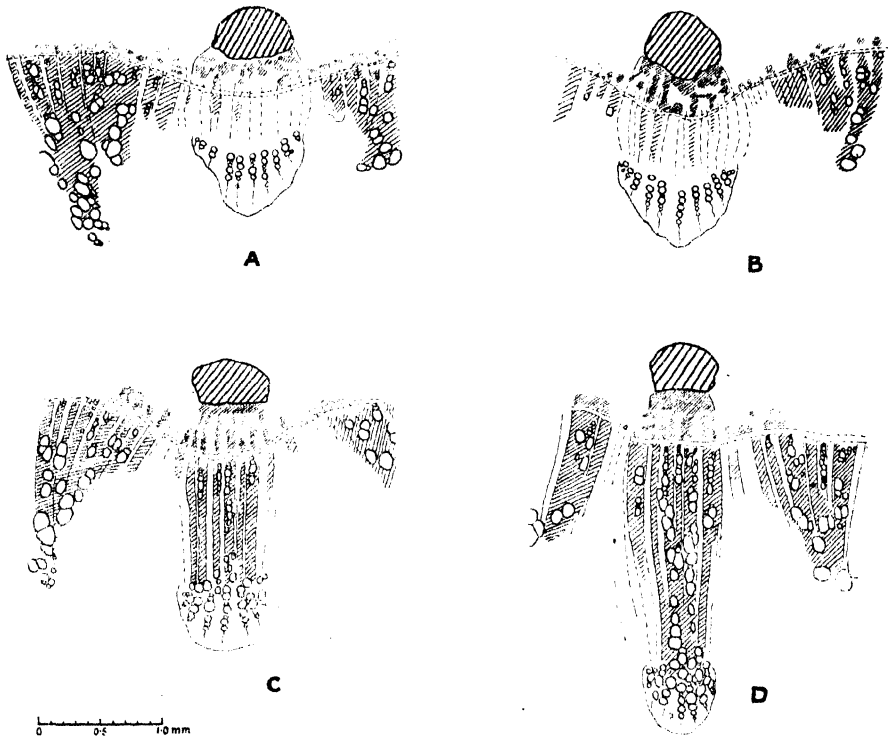


TEXT-FIG. 8. Sections of the median trace bundle of the leaf (already mature) at the seventh node of a vigorous plant: (1) in internode VII; (2) in internode V; (3) in internode IV. In the last, the small amount of primary xylem is supplemented by secondary xylem (cross-hatched) with large pitted vessels.

Farther down, where the primary growth of the parenchyma comes to an end while the leaf to which the bundle belongs is still expanding, the further growth of the bundle is affected and the additional xylem, although it is formed by the same cambium, develops the histological characters of secondary wood, with much larger pitted vessels, solid bands of fibres, and secondary medullary rays.

This formation of secondary xylem, once it has well begun, continues steadily and the cambium does not become dormant as in the internodes above. This is illustrated by Text-fig. 9, which represents bundles from one and the same section of an old stem. They are the median bundles belonging to leaves of successive nodes above the plane of section, and therefore of diminishing age; but the youngest, D, has formed far more xylem than the others. Here, too, it includes large pitted vessels which have been produced in normal proportion along with the wood fibres throughout the period of growth. In bundle C the secondary xylem is

less in amount and at first consisted mainly of fibres. Only later was the formation of vessels resumed, and these were small. Comparison with bundle D, and with adjoining regions, where in the latest xylem the vessels diminish to a corresponding size, suggests that the plant is coming to the end of its growth (the inflorescence was already expanded), so that the cambium of bundle C never awakened to full secondary activity. In bundle B the cambium has been still less active; it formed at first only



TEXT-FIG. 9. A-D. Median trace bundles at one level, belonging to leaves at four successive nodes above the plane of section, drawn from the section shown in Text-fig. 4 and Plate XVII, Fig. 5, illustrating the behaviour of the fascicular cambium at different distances from the leaves (see text). Wood fibres shaded.

parenchyma and then added a few bands of fibres but no vessels. In bundle A there is least secondary tissue and that is almost wholly parenchymatous.

Between corresponding parts of one leaf-trace bundle the contrast would be even greater.

Latest phase. In older, lower internodes later stages in the ontogenetic sequence are to be found, but on a smaller scale. Throughout the leaf-trace bundle the cambium ultimately awakens to full secondary activity and

gives rise to typical secondary wood with vessels, fibres, and medullary rays. By this time the tangential growth has ceased, the primary medullary rays have been bridged by cambium, and a uniform zone of wood is formed by radial activity of the continuous cambium, as in a typical woody stem, until the growth of the plant as a whole comes to an end (Plate XVII, Fig. 3).

From a morphological point of view this final resemblance to a woody stem cannot be regarded as a peculiarity of the basal region. What differentiates the latter from the upper part is the opportunity to reach this ontogenetic phase, an opportunity which is denied to the upper part by those physiological qualities of the plant as a whole which find expression in its limited life.

Just as the secondary growth in thickness of the upper part of the stem follows, so far as it goes, the same lines as that of the basal internodes, so also the primary growth of the latter and the development of the trace bundles of the lowest leaves take place in the same way as has been described for the upper part of the shoot.¹

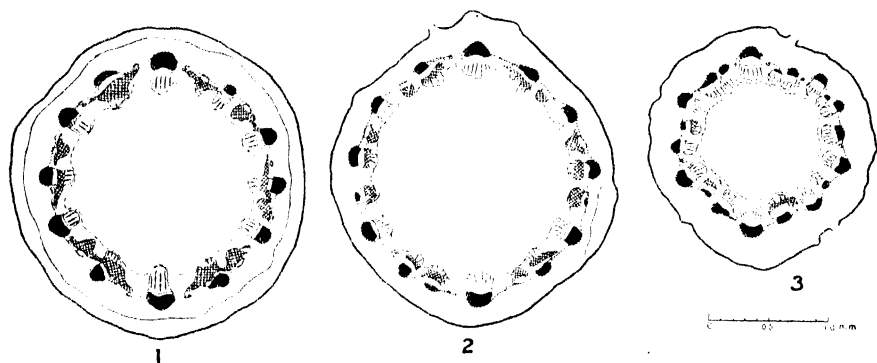
Thus throughout the shoot the same mode of organization is revealed. The key-note is plastic adaptability. The mode of growth of the primary bundles is equally suitable for the production of the small bundles of the young plant, when food supplies are limited, and of the large bundles of the full-grown plant, with its root system well established and its resources fully mobilized. The mode of secondary growth in thickness is especially suited to the stem of a large annual plant, as it secures the necessary rigidity with great economy, and because the growth of the lower parts is so intimately correlated with, and responsive to, the course of development above.

This correlation is further illustrated by the contrast between vigorous plants and small plants grown with their roots confined. A more extreme contrast is afforded by the meagre plants grown in sawdust and not transplanted. The mature plants are little more than a foot high, bear small few-rayed capitula, and have stems which even in the lower internodes are hardly thicker than those of the young plant. As Text-fig. 10 illustrates, there is little secondary growth even in the lowest internodes; additional rigidity is secured by the lignification of the perimedullary parenchyma. It is also of some interest that the decussate arrangement of the leaves persists till just below the inflorescence, five or six pairs being formed.

This example shows the effect of mere starvation on the structure of a species which under favourable conditions shows a considerable amount of

¹ The only apparent exception is the growth of the cotyledonary traces; but Chauveaud's seedling studies (*Ann. Sci. Nat., Bot.*, 9^e sér., xiii, 1911, and other papers quoted therein) suggest that 'xylem superposé' is cambial in origin. Cf. also Lenoir, *ibid.*, 1921.

secondary growth and produces a woody base closely resembling the stem of a woody plant. The approximation of the structure of the starved plant to that of an extreme annual is suggestive. With it may be compared Sinnott and Bailey's generalization¹ 'that an herbaceous stem, in all its



TEXT-FIG. 10. Sections of the stem of a starved dwarf plant already flowering. (1) First epicotylar internode (contrast Fig. 4, Plate XVII). (2) Third internode (cf. Text-figs. 1 and 2). (3) Sixth internode, near the capitulum. Endodermis dotted; the broken line indicates the boundary of the perimedullary lignified parenchyma; secondary wood cross-hatched (mainly fibres).

essentials, is like the first annual ring of its woody relatives'. It is not inconceivable that a shortening of ontogeny might gradually result from a change of climate or migration to a less favourable habitat through physiological effects analogous to starvation without any fundamental morphological change.

DISCUSSION.

Phylogenetic interpretations and ontogenetic stages. The structure of herbaceous stems has received a considerable amount of attention within recent years from anatomists who have sought evidences of the evolution of herbaceous from woody types and have endeavoured to interpret this evolution as a progressive adaptation to a shortened life-cycle.

One class of anatomical evidence adduced is that derived from a comparison of upper and lower regions of the same stem, on the assumption that the base retains ancestral features. Such a comparison does not by itself provide adequate data regarding the ontogeny of a stem, for each region has its own ontogeny. The method adopted in the present study of the Sunflower, of comparing the same region of the stem in similar individuals of different age, provides the means of testing, in a particular example, the validity of evidence of the kind referred to.

Jeffrey has emphasized the differences between the upper and lower

¹ Origin and Dispersal of Herbaceous Angiosperms. Ann. Bot., xxviii, p. 559, 1914.

regions of herbaceous stems and has applied the doctrine of recapitulation. For instance,¹ he points to the 'circular and complete woody cylinder' in the lower region of the epicotyl and the multifasciculate structure of the upper region of the same plant as 'developmental evidence' pointing to the 'derivation of herbaceous forms from woody ones'. Again, referring to *Helianthus hirsutus*, he says² that in the upper 'region the foliar traces, instead of being depressed below the level of the woody cylinder', as in the basal region, 'are outstanding, a condition very commonly found in extreme herbs which have largely lost their woody texture'.

Sinnott and Bailey³ similarly say, 'The base of most herbaceous stems is much stouter than the upper portion and often shows a close resemblance to a woody twig. On passing upward from such a base to the more delicate portions of the stem we can readily observe the progressive decrease in cambial activity and increase in parenchymatous tissue which have caused the development of the herbaceous type.' An application of the theory of recapitulation seems to be implied, though elsewhere Sinnott and Bailey show that they regard the theory as only to be applied with caution.

The points emphasized in the quotations are: (1) the woodiness of the base and the decrease in cambial activity upwards; (2) the depression of the leaf-trace bundles in the lower region and their outstanding position in the upper region; (3) the increasing proportion of parenchymatous tissue upwards.

With regard to (2), Text-fig. 2 shows that in the lower internodes of *Helianthus annuus*, as well as in the upper, the median leaf-trace bundles are at first outstanding, in conformity with the decurrent leaf bases. This is the case even in the first epicotylar internode. They become depressed owing to the vigorous secondary growth of the segments on either side and the sluggishness of their own cambium. The more conspicuous prominence of the trace bundles in the upper part is an expression of their precocity and more vigorous growth. Even here their prominence diminishes as the stem grows in diameter, and, when secondary growth proceeds far enough, they become depressed as at the base of the stem.

Concerning (1), the greater woodiness of the base, it is obvious that as the lowest internodes are the oldest they have had most time in which to produce secondary wood. In the case of *H. annuus* a comparison of the ontogeny of the upper and lower internodes reveals no fundamental difference in mode of organization. There is merely a change of scale, reflecting the increasing vigour of primary growth,⁴ and naturally in successively younger internodes less and less secondary wood. Secondary growth, so far as it goes, always follows the same course and shows the same distribution. At the

¹ Anatomy of Woody Plants, Chicago, 1917, p. 191.

² Ibid., p. 403.

³ Origin and Dispersal of Herbaceous Angiosperms. Ann. Bot., xxviii, p. 559, 1914.

⁴ In starved dwarf plants this increase of scale does not occur. See Text-fig. 10 (3).

very base the somewhat greater thickness of secondary wood than corresponds to mere age can be interpreted as an accommodation phenomenon appropriate to the transition from the tangentially expanding stem to the pithless, radially thickening root.

As regards (3), if upper and lower regions are compared at corresponding stages of their ontogeny, there is no clear evidence of an increasing *proportion* of parenchyma upwards. Moreover, in any given region, after primary expansion is complete, the proportion of functional parenchyma diminishes because of the increasing amount of secondary wood and the collapse of the pith. The real differences, therefore, between the upper and lower regions of the stem of *Helianthus annuus* are reduced to (a) a difference of scale and (b) a difference of age and ontogenetic stage. Neither of these differences is fundamentally morphological. The morphological basis is the same throughout. Nor does either of these differences appear to justify an application of the theory of recapitulation. All that can be said is that, just as the Sunflower is shorter lived than a tree, so the upper part of a Sunflower is shorter lived than the base. All that remains as anatomical evidence for the derivation of this herbaceous stem from a woody form is simply the presence of a cambium which gives rise to woody tissues similar to those which characterize woody stems.

The *increase of scale* in the upper part of the shoot calls for some further consideration. Closely correlated with it is the secondary growth in thickness of the parts below by a method which allows of a rapid increase in diameter and at the same time is economical. Thus the necessary increase in rigidity keeps pace with the expansion above without drawing as heavily on the available food supplies as if the same increase in strength were secured entirely by radial growth, like that of a woody twig. A larger proportion of the food is therefore available for the young developing organs. The increasingly vigorous primary growth of these, which is reflected in the increase of scale, must involve a great expenditure of food material. Translocation must be proportionately efficient. Since Sachs's experiments *Helianthus annuus* has been well known as a plant which both assimilates and translocates the products of assimilation with remarkable rapidity. Recently Willstätter and Stoll¹ have demonstrated that this species, along with certain other vigorous herbaceous plants, owes its capacity for rapid assimilation, not to a greater content of chlorophyll, but to greater efficiency of the chloroplasts themselves. It may be suggested that for the maintenance of this efficiency translocation must be correspondingly efficient so that accumulation of products is avoided.²

¹ Untersuchungen über die Assimilation der Kohlensäure, Berlin, 1918, p. 85, &c.

² Further light may be expected on this point from Briggs, Kidd, and West's quantitative studies of growth in *Helianthus*; cf., for instance, their reference to the possibility that the utilization of assimilable material, governed by temperature, might control assimilation. *Annals of Applied Biology*, vii, 1920, p. 217.

It has been shown how at the node and elsewhere there is anastomosis of phloem, independently of the xylem, so that a minimum path to the shoot apex is secured for at any rate a part of the products entering at the node. Whether in this respect the Sunflower is exceptional or whether by any other structural features translocation is specially facilitated are questions requiring comparative investigation.¹

Whatever the answers to these questions may be, the fact remains that the rapid expansion of the shoot necessarily implies the rapid translocation of increasing supplies of food materials from the assimilating leaves. While expenditure of food increases with increase in the available supply, there seems no reason to expect increased storage. The emphasis laid by Jeffrey and by Sinnott and Bailey on the greater provision of storage tissue in herbs appears, therefore, to be exaggerated.

Direct evidence on this question is incomplete, but it may be noted that although starch appears in abundance in the leaf of the Sunflower as a transitory reserve, it is absent from the stem, except in the endodermis, and little inulin crystallizes out in alcohol.

It is also to be remembered that storage is not the only function proper to parenchyma, but that it plays a very important part in the mechanics of primary growth, especially in elongation.

Secondary parenchyma. Jeffrey has laid special stress on the *parenchymatization* of the secondary xylem of the foliar traces as providing additional storage tissue. Here again there is no direct evidence that this secondary parenchyma is in the Sunflower specially concerned with storage. In any case there are other points of view from which it may have significance.

In the first place the secondary tissue in the leaf-trace bundle not only becomes more parenchymatous upwards, but also diminishes in amount till at the node it is negligible. The mechanical aspect of this gradation is probably of considerable importance. If the cambium were radially active immediately below the node the primary xylem in the stem would be separated from the leaf base, and in the outgoing portion the vessels would be put out of action. Instead of this, the strain is evenly distributed and the primary xylem strand slopes gently outwards towards the point of departure from the vascular zone. The strain is not, however, wholly removed. Occasionally the secondary parenchyma just outside the primary xylem is stretched, like the pith within, and the primary xylem is isolated from the later fibrous secondary xylem. In such case the presence of yielding parenchyma is clearly of mechanical advantage, as well as the collenchy-

¹ It is, of course, unlikely that the vigour of assimilation and growth depend wholly on structural features; e.g. Molisch (*Mikrochemie der Pflanzen*, 1921, p. 91) names the genus *Helianthus* among 'nitrate plants', and with this and other herbaceous genera named as rich in nitrates contrasts trees as generally poor in nitrates.

matous sheaths that surround the vessels and hold the primary xylem together. Ultimate partial or complete failure of accommodation to mechanical strains may account for the fact that the lower leaves wilt more readily, although they are nearer the water-supply, and finally shrivel.¹

Correlations and their causal interpretation. Secondly, the variations in degree of activity of the cambium in the leaf-trace bundle and elsewhere demand, and should ultimately be susceptible of, a causal explanation. A brief consideration of the observed correlations from this point of view may perhaps be not unprofitable.

The sluggishness of the cambium in the upper part of the leaf-trace bundles is sufficient evidence that the greater cambial activity elsewhere is not determined by an abundance of food.

The sectors which show vigorous tangential growth are synthetic traces always in communication with young expanding leaves near the apex. There is a close correlation between the growth of these sectors and the expansion of the shoot as a whole.

The growth of the independent leaf-trace bundle is, on the other hand, closely correlated with the expansion of the leaf it serves, and tends to cease when the leaf has reached its full size.

In the lower part of the bundle, however, where primary growth comes to an end before the leaf is fully expanded, the primary xylem is supplemented by secondary xylem. It is an interesting fact that this secondary growth does not cease when the growth of the leaf comes to an end, but continues until the flowering season brings a diminution in the rate of growth of the stem as a whole. Thus, although at its initiation this secondary growth is correlated with the growth of the leaf, after initiation it ceases to show the same correlation, but is correlated instead with the growth of the plant as a whole.

The upper parts of the bundle reach their full size before primary growth has ceased around them. Secondary growth is then only slowly resumed, and the tissues produced reflect qualitatively as well as quantitatively the relative inactivity of the cambium, which is the more pronounced the nearer the node. From this point of view the so-called 'parenchymatization' of the xylem may mean simply arrested differentiation.

In the final stage of the resumption of activity, the cambium throughout the bundle produces typical secondary wood and behaves uniformly with the rest of the cambium at the same level, in correlation with the upper part of the shoot. This stage, however, is only represented in the older part of the stem, and is probably reached only when the leaves have fallen.

These correlations strongly suggest that each leaf has a sphere of influence in the vascular zone which is localized tangentially and longi-

¹ Cf. Thoday, Proc. Roy. Soc., B., lxxxii, 1909, p. 25.

tudinally.¹ So long as a leaf is growing, its influence is felt throughout the bundles of its leaf-trace and the synthetic traces with which these are continuous below. The synthetic traces come under the combined influence of a continual succession of expanding leaves. The upper part of a leaf-trace, on the other hand, follows the lead of its own leaf, and its growth practically ceases when the leaf is fully expanded. The lower part commences to form secondary xylem under the influence of its own leaf, but this change from primary to secondary growth apparently involves a transference from the sphere of influence of the leaf into that of the apex. After the growth of the leaf has ceased the influence of the apex gradually encroaches still farther upwards until it controls the activity of the cambium throughout the bundle.

It seems necessary also to suppose that the sphere of influence of the leaf extends to the adjoining parenchyma of the medullary rays that flank the trace bundles and inhibits meristematic activity there; for otherwise it is difficult to understand the fact that, in spite of the active extension of the cambium from the synthetic trace on the other side of a ray, the complete bridging of the ray by cambium, and still more by secondary wood, is so long delayed. This assumption gives a more precise meaning to the localization of the leaf-trace bundles in this multifasciculate stem, and also covers the persistence of the primary medullary rays which is a necessary condition of its specialized tangential mode of growth in thickness.

The view of the observed correlations here tentatively outlined is at least a useful working hypothesis. It suggests a field for experimental investigation in the study of the effects of removing leaves, at various stages of their development, on the growth of the trace bundles at different levels.

In conclusion, it may be pointed out that plants were intentionally selected for their freedom from lateral branches, which would have added further complications. A study of the way in which branches are accommodated and their influence on the main stem in this plant would not be without interest.

The only other species which has been examined for comparison is *Helianthus tuberosus*. The aerial stem of this species shows similar tangential growth, similarly distributed.

As regards the cambial origin of the primary xylem, this is probably a widespread phenomenon among Angiosperms. The absence of a sharp line of demarcation between primary and secondary wood has often been remarked upon. A casual glance at the first annual ring of the Oak and

¹ If the correlations on which Miss Saunders has based her 'Leaf-skin Theory' (Ann. Bot., xxxvi, p. 135, 1922) are to be similarly interpreted, they imply a delimitation of spheres of influence in the superficial tissues on a plan very different from that which governs their delimitation in the vascular zone.

other woody plants is sufficient to make the cambial origin of the whole of the xylem in them also highly probable.

Mrs. Arber's studies of Monocotyledons,¹ on the one hand, and Chauveaud and, more recently, Lenoir's investigations of seedling ontogeny² on the other, point largely in the same direction. But on this question more definite data are desirable.

SUMMARY.

1. The primary and secondary growth and differentiation of the stem of *Helianthus annuus* are described. The principal method employed was to compare the dimensions and structure of corresponding regions of different age and size.

2. In the primary differentiation of the leaf-trace bundle the small procambial strand is early distinguishable into an outer group of fibre initials and an inner procambial crescent which grows laterally. The whole of the xylem originates from a cambium which arises very near the inner margin of the crescent and extends tangentially in both directions *pari passu* with it. The bulk of the procambial crescent differentiates as phloem, beginning in the median region while the flanks are still meristematic and growing actively.

3. The primary growth of the stem is illustrated, including the interpolation of new bundles in the widening intervals between the precocious primary bundles. The transverse growth of a leaf-trace bundle is correlated with that of the leaf and not with that of the internode as a whole.

4. At the node the entering leaf-trace bundles are accompanied by strands of phloem which connect laterally with the phloem of adjoining bundles. Anastomosis of phloem strands is also frequent elsewhere.

5. Secondary growth in thickness is at first due to the activity of the cambium in the 'synthetic traces' that communicate directly with the upper still expanding leaves. This activity is in parts tangential as well as radial. Extensions of the cambium are also formed in the adjoining medullary rays, which widen to accommodate them. As a result of this tangential growth in the cambial region the pith is distended and often becomes hollow.

6. The cambium in the upper part of the mature leaf-trace bundles is meanwhile inactive and only gradually resumes active growth. The secondary xylem is at first largely parenchymatous. Towards the base of the stem a later stage of ontogeny is reached in which the fascicular cambium forms secondary wood in no way differing from that formed elsewhere.

7. The mode of growth, both primary and secondary, is the same from the basal internode throughout the stem. The differences are due to the larger scale of the primary tissues in the upper part and to the fact that the

¹ Ann. Bot., xxxvi, p. 251, 1922, and other papers there cited.

² Loc. cit.

upper region represents an earlier phase of ontogeny than the woody base. The validity of Jeffrey's application of the theory of recapitulation to such differences is questioned.

8. The stems of small starved plants form very little secondary tissue even at the base, and their structure suggests comparison with smaller and shorter-lived annuals.

9. Various interpretations of the facts are discussed.

EXPLANATION OF PLATE XVII.

Illustrating Professor Thoday's paper on the Stem of the Sunflower.

The figures are reproduced from photographs of transverse sections.

Fig. 1. One of the two principal median leaf-trace bundles at the middle of the first epicotylar internode of a young plant, showing the continuity of the rows of elements of the primary xylem through the cambial zone.

Figs. 2 *a*-2 *f*. Sections of an old stem photographed about natural size in oblique illumination, with a dark background to show up the wood.

a, hypocotyl, about 1.5 cms. below the cotyledonary node.

b-*e*, first epicotylar internode: *b*, just above the cotyledonary node; *c*, 1 cm., *d*, 2 cm., *e*, 8 cm. above the node.

The series *a*-*e* illustrates the increasing distension of pith and diminishing thickness of the woody zone upwards (see p. 491).

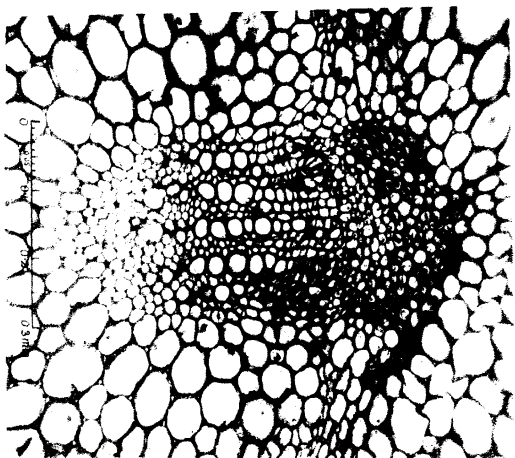
f, at a higher level, in the region of transition to two-fifths phyllotaxy.

Fig. 3. Same as 2 *b*, $\times 4$. Base of first epicotylar internode, showing continuous zone of wood. The older wood, next the pith, is not continuous, and this together with the distension of the pith is evidence of an earlier tangential phase of growth. This has now been superseded by the later phase in which fascicular and interfascicular cambium behave uniformly and growth is radial, as in a woody twig.

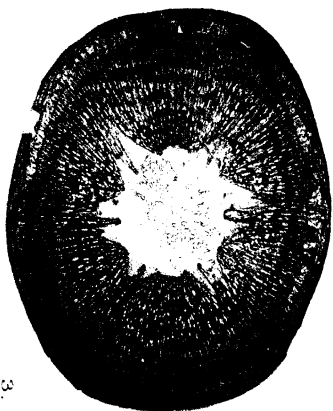
Fig. 4. Same as 2 *e*, $\times 4$. Upper part of same internode. Tangential growth is about at an end. The principal bundles are separated by broad wedges of secondary wood in which the divergence of the secondary medullary rays is evidence of tangential growth. The distended pith shows clear signs of strain.

Fig. 5. Same as 2 *f*, $\times 4$. The number of bundles is larger, but tangential growth is still exhibited between the principal ones. The pith has given way in the middle and the outer intact zone shows evidence of strain.

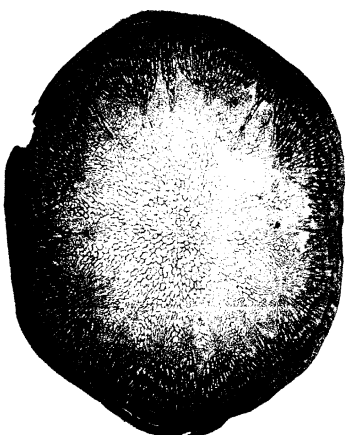
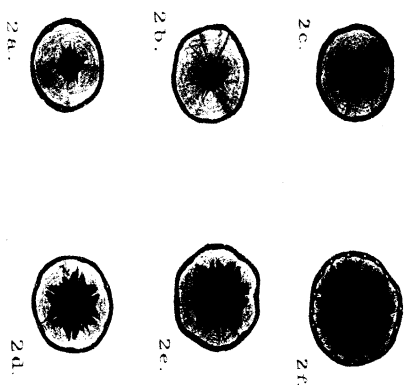
Fig. 6. A part of the same section more highly magnified. The two bundles near the ends of the photograph are the median and one of the lateral trace bundles belonging to the leaf at the second node above the section. They show the parenchymatous nature of the secondary xylem formed at first by the cambium in the upper part of the leaf-trace bundles.



1.



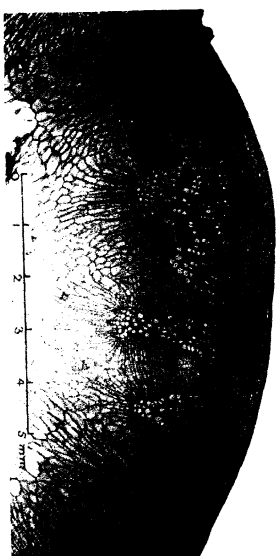
3.



4.



5.



6.

On the Course of Absorption and the Position of Equilibrium in the Intake of Dyes by Discs of Plant Tissue.

BY

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With eight Figures in the Text.

INTRODUCTION.

THE problem of the absorption of dyes by plant tissue has been the subject of much investigation, especially of late years, but few quantitative data are available. One of the first investigators to examine the problem was Pfeffer (4), whose researches established the fact that many dyes, especially those known as 'intra-vitam stains', are absorbed from very dilute solutions by plant tissue and accumulate in the cells, either as a soluble compound in the cell sap, or as a solid precipitate; various substances in the living cell render the accumulation of the dye possible, the best known being tannin and phloroglucin, which form 'non-diosmosing' compounds with the dyes. Pfeffer also demonstrated the fact that normal growth continues provided that the external solution is sufficiently dilute.

Later workers confirmed many of Pfeffer's conclusions, and established the fact that a larger proportion of dye is absorbed from more dilute solutions. The explanation which was usually accepted was that the dye diffused through the 'plasma membrane' by a process of osmosis, and that the concentration gradient was maintained by the formation of non-diosmosing compounds, as described by Pfeffer.

Recently, however, Moore and his co-workers (3) have challenged the theory that the entrance of substances into the cell can be explained as a process of osmotic diffusion through a semi-permeable membrane; they explain the powers of selective absorption shown by the cell by the supposition that 'the cell protoplasm has selective adsorptive powers for different ions, and that such ions exist in the cell in combination or adsorption with the cell substance'.

Stiles and Kidd (6), in experiments on the influence of external concen-

tration on the intake of salts by plant cells, found that the relation between final internal concentration and final external concentration was given by the adsorption equation $y = kc^{\frac{1}{m}}$, where y = final internal concentration, c = final external concentration, and k and m are constants. They declined to put forward any proposals as to the mechanism of salt intake by the cell, on the ground that the data available were insufficient to justify any conclusions.

It is a well-known fact that many substances, e.g. charcoal, form adsorption compounds with dyes and will decolorize a solution of dye with which they are in contact.

The present investigation was undertaken in order to obtain quantitative data as to the course of absorption of dyes by plant tissue, and also to determine whether the results would furnish any evidence in favour of the adsorption theory of absorption put forward by Moore and Roaf.

METHODS.

The method of experimentation employed was similar to that elaborated by Stiles and Jørgensen in their 'Studies in Permeability' (7). Storage tissue of various species was used; in practice it was found that the best results were obtained with carrot tissue, and accordingly this tissue was employed in most of the experiments, though potato, artichoke, and turnip were also used. Cylinders of tissue were cut out by means of a cork-borer, 2 cm. in diameter; discs 1 mm. in thickness were cut with a hand microtome. The discs were thoroughly mixed and washed in two or three changes of distilled water; they were then weighed in sets of two or four, and immersed in 50 c.c. of the experimental liquid, in corked bottles. The discs were weighed at intervals, in order that evidence might be obtained as to whether the tissue was in a healthy condition, or was injured by the dye. The concentration of the dye in the external solution was estimated colorimetrically; each experiment was performed in duplicate.

The observations were continued in each experiment until equilibrium was established, or until the colour of the dye in the external solution was so changed that a comparative estimation of the concentration was impossible.

A number of water-soluble dyes were used, and in each case four concentrations were employed—0.1 per cent., 0.05 per cent., 0.01 per cent., 0.005 per cent.

In addition to experiments with living tissue, a few series of experiments were carried out with tissue which had been killed in a mixture of absolute alcohol and acetic acid before immersion in the experimental solution. A few experiments were also made to determine the influence of temperature on the absorption of dyes.

EXPERIMENTAL RESULTS.

Series I. *Experiments with living tissue.*

Discs of living tissue, prepared as described in the preceding section, were immersed in solutions of the following water-soluble dyes: neutral red, methylene blue, methyl violet, aniline blue, eosin, and Congo red.

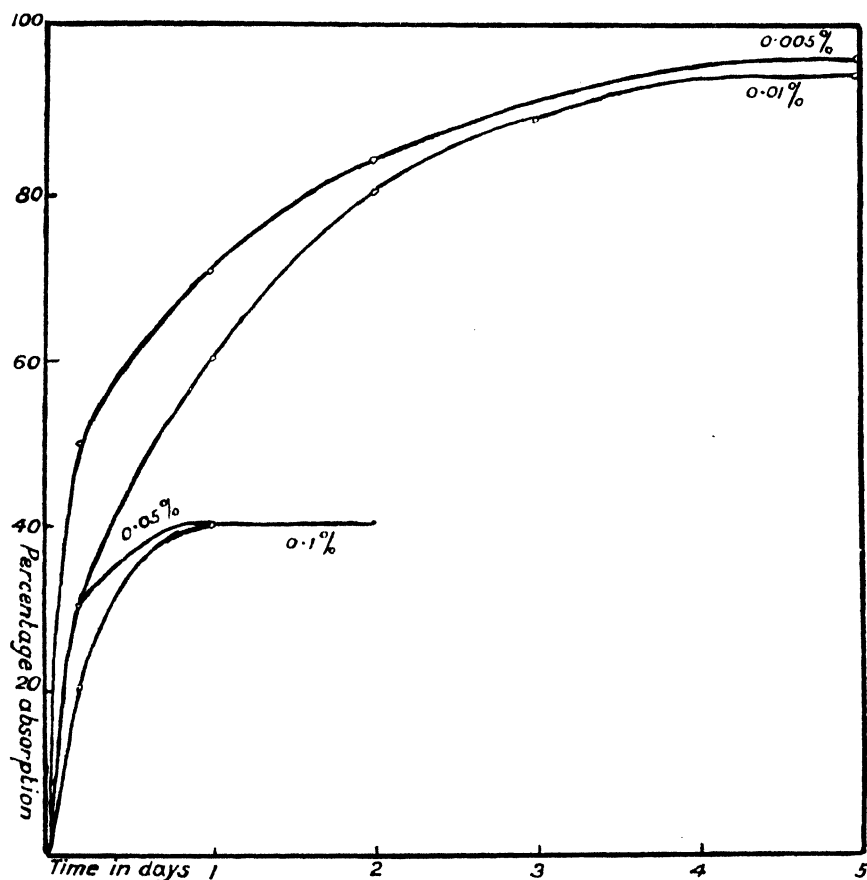


FIG. 1. Course of absorption of neutral red, by living carrot tissue, from solutions of various concentration.

The concentration of dye in the external solution was estimated at intervals, and the tissue was weighed.

The results were similar in the case of all the basic dyes employed except aniline blue; the latter dye was not absorbed in appreciable quantity from the more concentrated solutions, and the amount absorbed from the more dilute solutions was small in comparison with the other basic dyes. The results obtained with the acid dye, eosin, resembled those obtained with aniline blue, while Congo red, the only other acid dye used, was not

absorbed in appreciable quantity from any concentration. In the cases of aniline blue and eosin, equilibrium was attained very rapidly, and no curves of absorption could be plotted.

In Table I the results obtained with neutral red are summarized, and the curves of absorption are plotted in Fig. 1.

In Tables II and III the final external concentrations of all the dyes used are given.

The numbers quoted for the internal concentration are proportional to those obtained for the external concentration, and do not represent absolute quantities; they are strictly comparable among themselves, however, as approximately the same amount of tissue was used in every case. The actual internal concentration is much greater, as the internal volume of solution is very small.

TABLE I. *Absorption of Neutral Red, by Discs of living Carrot Tissue, from Solutions of Different Concentrations.*

<i>Duration of experiment in hours.</i>	<i>External concentration in per cent.</i>	<i>Relative internal concentration.</i>	<i>Weight in grm.</i>
0	0.1	0.0	1.04
5	0.08	0.02	0.86
24	0.06	0.04	0.81
48	0.06	0.04	0.78
0	0.05	0.0	0.87
5	0.035	0.015	0.75
24	0.03	0.02	0.68
48	0.03	0.02	0.67
0	0.01	0.0	0.96
5	0.007	0.003	0.93
24	0.004	0.006	0.92
48	0.002	0.008	0.90
72	0.0009	0.0091	0.90
96	0.0007	0.0093	0.89
144	0.0005	0.0095	0.87
0	0.005	0.0	1.06
5	0.0025	0.0025	1.06
24	0.0015	0.0035	1.06
48	0.00085	0.00415	1.05
72	0.0004	0.0046	1.06
96	0.000225	0.00475	1.05
144	0.000125	0.004875	1.05

At the end of the experiments in this series a slight yellowish tinge was observed; this showed that the external solution was becoming acid.

TABLE II. *Absorption of Dye, by living Carrot Tissue, from Solutions of Various Concentrations.*

<i>Original external concentration in per cent.</i>	<i>Neutral red.</i>	<i>Final external concentration in per cent.—</i>				
		<i>Methylene blue.</i>	<i>Methyl violet.</i>	<i>Aniline blue.</i>	<i>Eosin.</i>	
0.1	0.06	0.032	0.03	0.1	0.1	
0.05	0.03	0.012	0.01	0.045	0.045	
0.01	0.0005	0.0008	0.0018	0.008	0.0085	
0.005	0.000125	0.0003	0.001	0.003	0.004	

TABLE III. *Absorption of Dye, by living Tissue of other Species, from Solutions of Various Concentrations.*

Original external concentration in per cent.	Final external concentration in per cent.—		
	Neutral red, Potato.	Methylene blue, Artichoke.	Methyl violet, Turnip.
0.1	0.085	0.04	0.08
0.05	0.018	0.007	0.03
0.01	0.0019	0.0027	0.0025
0.005	0.0008	0.0006	0.001

If the relation between the final external concentrations and the final internal concentrations corresponds to the adsorption equation $y = kc^{\frac{1}{m}}$, where y = final internal concentration, c = final external concentration, and k and m are constants, then the graph obtained by plotting the logarithm of

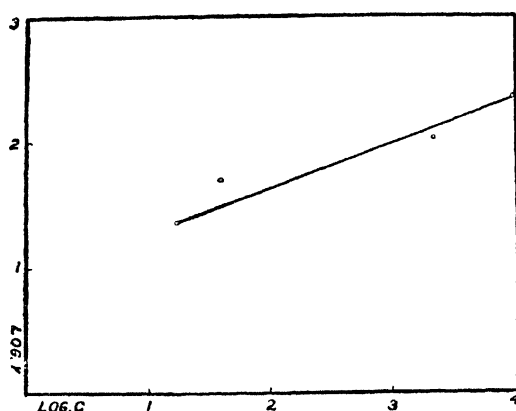


FIG. 2. The relation between final internal and final external concentrations in the case of living carrot tissue immersed in solutions of neutral red.

the final external concentration against the logarithm of the final internal concentration will approximate to a straight line. This was the case in most of the experiments with carrot tissue; in Figs. 2 and 3 the graphs for neutral red and methylene blue are so plotted. In the case of tissues other than carrot, however, the results differed somewhat; Fig. 4 shows the graph for neutral red, in which discs of potato tissue were immersed. Similar figures were given by the results obtained with artichoke and turnip tissue.

Series 2. *Experiments with dead tissue.*

These experiments were carried out with discs of carrot tissue, which were killed in a mixture of acetic acid and absolute alcohol; they were then thoroughly washed and transferred to the experimental dye solutions. Two sets of experiments were made, one with methylene blue and one with methyl violet. The results with methylene blue are summarized in Table IV, and the course of absorption is plotted in Fig. 5.

TABLE IV. *Absorption of Methylene Blue, by Discs of Dead Carrot Tissue, from Solutions of Various Concentration.*

<i>Duration of experiment in hours.</i>	<i>External concentration in per cent.</i>	<i>Relative internal concentration.</i>	<i>Weight of tissue in grm.</i>
0	0.1	0.0	0.71
24	0.078	0.022	0.72
48	0.073	0.027	0.71
96	0.065	0.035	0.70
0	0.5	0.0	0.90
24	0.033	0.017	0.91
48	0.025	0.025	0.90
72	0.020	0.030	0.89
0	0.01	0.0	0.73
24	0.0073	0.0027	0.73
48	0.0052	0.0048	0.73
72	0.0032	0.0068	0.73
120	0.0022	0.0078	0.72
0	0.005	0.0	0.70
24	0.0037	0.0013	0.76
48	0.0021	0.0029	0.75
72	0.0012	0.0038	0.75
120	0.0009	0.0041	0.74

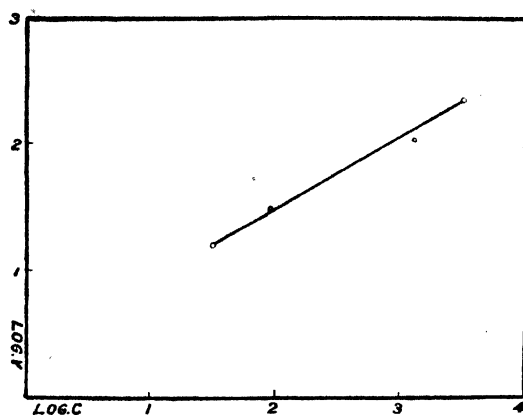


FIG. 3. The relation between final external and final internal concentrations in the case of living carrot tissue immersed in solutions of methylene blue.

Similar results were obtained with methyl violet, though the proportion of dye absorbed by the tissue in this case was slightly less. The final external concentrations for both dyes are set out in Table V, and in Fig. 6 the logarithm of the final external concentration is plotted against the logarithm of the final internal concentration in the case of methylene blue.

TABLE V. *Absorption of Dye, by Dead Carrot Tissue, from Solutions of Various Concentrations.*

<i>Original external concentration in per cent.</i>	<i>Final external concentration in per cent.—</i>	
	<i>Methylene blue.</i>	<i>Methyl violet.</i>
0.1	0.065	0.065
0.05	0.02	0.025
0.01	0.0022	0.003
0.005	0.0009	0.0013

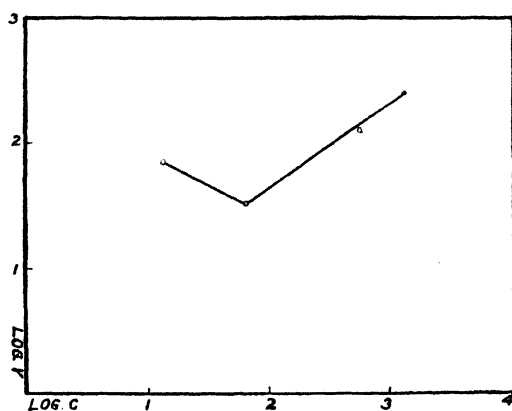


FIG. 4. The relation of final external and final internal concentrations in the case of potato tissue immersed in solutions of neutral red.

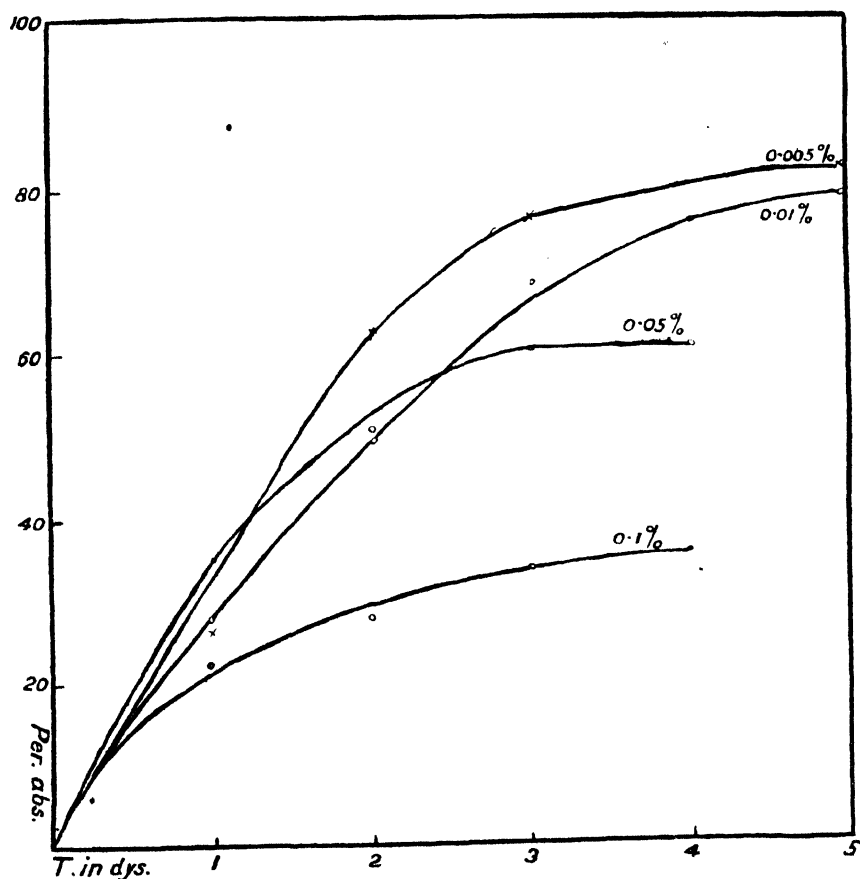


FIG. 5. Absorption of methylene blue from solutions of various concentrations by dead carrot tissue.

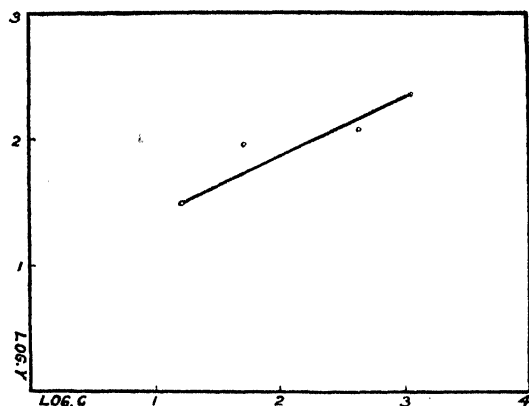


FIG. 6. Relation of final internal and final external concentrations in the case of dead carrot tissue immersed in solutions of methylene blue.

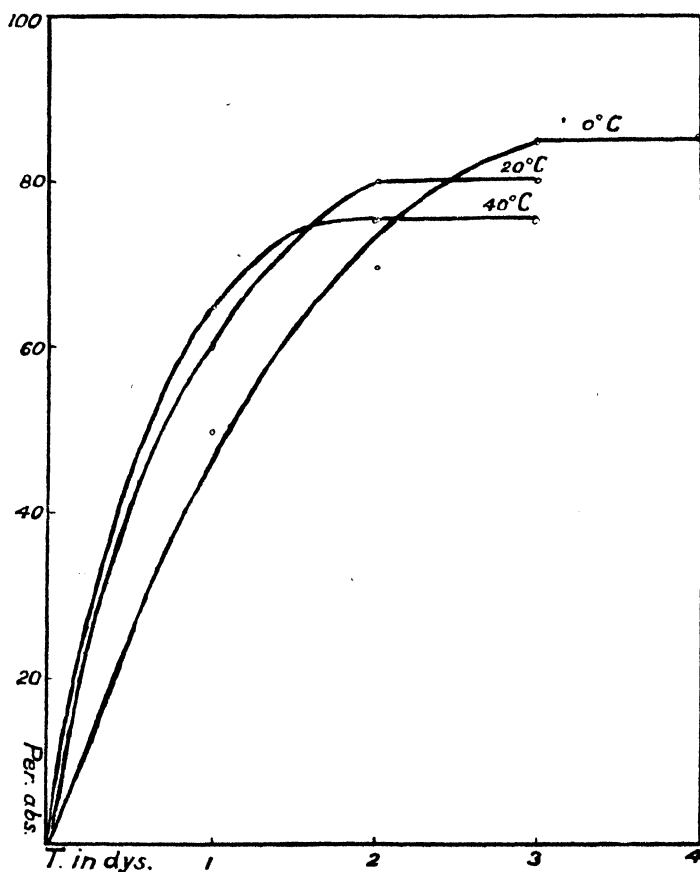


FIG. 7. Absorption of methylene blue from 0.01 per cent. solution by living carrot tissue at different temperatures.

Series 3. *Experiments to test the effect of temperature.*

Series of experiments were carried out, in which the bottles containing the experimental solutions were kept at 40° C., 20° C., and 0° C. The dye

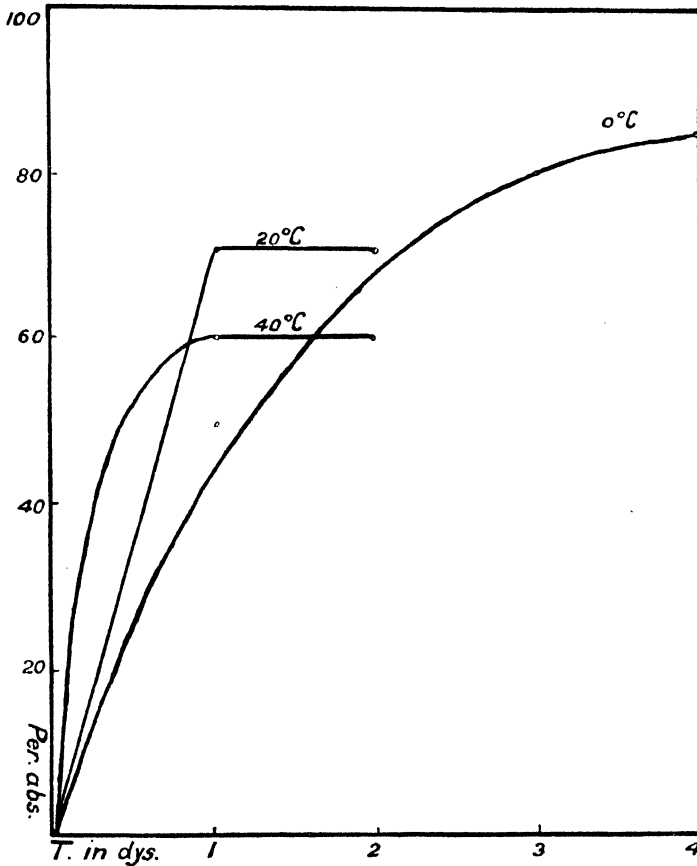


FIG. 8. Absorption of methylene blue from 0.005 per cent. solution by living carrot tissue at different temperatures.

used was methylene blue, and the experiments were made with living carrot tissue. The results are summarized in Table VI and Figs. 7 and 8. Similar results were obtained in a series of experiments with methyl violet.

TABLE VI. *Absorption of Methylene Blue from Solutions of Different Concentrations by Discs of Carrot Tissue at Different Temperatures.*

Temperature.	Original external concentration in per cent.	External concentration in per cent. after—		
		24 hrs.	48 hrs.	72 hrs.
40° C.	0.1	0.07	0.06	—
	0.05	0.025	0.02	—
	0.01	0.0035	0.0025	—
	0.005	0.002	0.002	—

TABLE VI (*continued*).

<i>Temperature.</i>	<i>Original external concentration in per cent.</i>	<i>External concentration in per cent. after—</i>		
		<i>24 hrs.</i>	<i>48 hrs.</i>	<i>72 hrs.</i>
20° C.	0.1	0.07	0.07	—
	0.05	0.03	0.02	—
	0.01	0.004	0.002	—
	0.005	0.0015	0.0015	—
0° C.	0.1	0.07	0.06	0.055
	0.05	0.02	0.015	0.015
	0.01	0.005	0.003	0.0015
	0.005	0.0025	0.002	0.001

DISCUSSION.

1. *Course of absorption.*

The absorption curves obtained in this investigation are similar to those obtained by Stiles and Kidd (6) for the intake of salts from solution by plant tissue—that is, the curves rise steeply at first, but gradually become more horizontal as the condition of equilibrium is approached.

The curves also show that the condition of equilibrium is reached much more rapidly with more concentrated external solutions than is the case with more dilute external solutions. This might be expected as the effect of the dye is much more harmful in concentrated solutions than in dilute solutions, as is shown by the rapid loss of weight by the discs in the case of the former. This loss of weight indicates loss of turgor.

2. *Influence of the acidity or basicity of the dye employed.*

The majority of the dyes used in this investigation are basic; only two acid dyes were employed, viz. Congo red and eosin. The first of these was not absorbed in appreciable quantity by the plant tissue from solutions of any concentration, and the external solutions became darker in colour, presumably owing to reactions taking place between the dye and substances which diffuse out from the cells. In the case of eosin, the dye was not appreciably absorbed from the more concentrated solutions, and only to a slight extent from the more dilute solutions.

The basic dyes, on the contrary, with one exception (aniline blue), were rapidly absorbed, and when equilibrium was reached the internal concentration was frequently greater than the external concentration, especially in the case of the more dilute solutions.

These results are in accord with the earlier ones of Ruhland (5), who found that basic dyes were rapidly absorbed, while acidic dyes were, at most, absorbed to a very slight extent. The results also agree with those of Collander (1) in his recent work on the sulphonic acid dyes. He found that these dyes were absorbed in relatively slight amounts by plant tissue. In an extensive series of experiments, he estimated the ratio between the

internal concentration and the external concentration of the dye when equilibrium was reached. He found that in many cases the ratio was of the order

$$\frac{x}{y} = \frac{1}{120}, \text{ or } \frac{1}{240},$$

or even less, where x = the final internal concentration, and y = the final external concentration.

3. *Influence of the size of the molecule.*

The dyes which were employed in the present investigation may be classified with regard to the size of the molecule, as follows:—

<i>Colloid</i>	<i>Semi-colloid</i>	<i>Crystalloid</i>
Congo red	Methyl violet	Methylene blue
Aniline blue	Neutral red	Eosin

It is evident, therefore, that in the case of crystalloids and semi-colloids, the size of the molecule has little influence in determining the amount of the dye absorbed; this is in agreement with the earlier conclusions reached by Ruhland (5), though in his later work he completely altered his attitude. Neither of the dyes classed as colloids, however, was absorbed in any appreciable quantity; the case of aniline blue is especially interesting, as this dye is basic, and was the only basic dye investigated which was not rapidly absorbed. It is noteworthy that Pfeffer recorded the fact that aniline blue is not absorbed by plant tissue.

4. *Comparison of the results obtained with living and dead tissue.*

If the results recorded in Table II are compared with those in Table V, it will be seen that the discs of dead tissue behaved in a very similar manner to the discs of living tissue. In every experiment the absorption was slightly less in the case of dead tissue; however, in view of the low degree of accuracy obtainable with the colorimetric method used, and also in consideration of the variability of plant tissue, it is probable that this difference cannot be regarded as significant.

It is a possibility that with discs of living tissue the dye is chiefly absorbed by the superficial dead cells, but examination of sections under the microscope showed that the dye penetrated to the internal living cells.

5. *Effect of temperature.*

As only a few experiments were made to test the effect of temperature, no definite conclusions can be drawn. It is interesting to note, however, that the percentage absorption of the dye is increased with decrease of temperature; this is especially marked in the case of the more dilute solutions. This effect of temperature is characteristic of adsorption processes as distinct from chemical combinations.

6. *Influence of the concentration of the external solution.*

The results given above show that the percentage absorption of the dye is increased as the external solution becomes more dilute. This is very clearly shown in the case of the basic dyes, which were absorbed from all concentrations; in the case of the acid dyes and aniline blue, if there was any appreciable absorption it only took place in the more dilute solutions.

When the logarithm of the final external concentration is plotted against the logarithm of the final internal concentration the resulting graph approximates to a straight line, in the case of most of the basic dyes and eosin. This indicates that the relation between these concentrations corre-

sponds to the adsorption equation $y = kc^{\frac{1}{m}}$, where y = final internal concentration, c = final external concentration, and k and m are constants. Although this fact cannot be taken as proof that the absorption of dyes by plant tissue is a process of adsorption, yet it may be regarded as evidence in favour of the view that adsorption plays an important part in the absorption process; the results obtained in the few experiments carried out at different temperatures support this view. In several cases, however, notably with tissue other than carrot, the process seems to be complicated by some other reaction, possibly one of chemical combination.

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The Significance of the 'Foliar Ray' in the Evolution of Herbaceous Angiosperms.

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With Plates XVIII and XIX and one Figure in the Text.

IN 1914 the writers¹ presented in this Journal a body of evidence in support of the suggestion of Hallier,² of Eames,³ and of others that herbaceous angiosperms are derived from arborescent or fruticose ancestors. In that paper we not only discussed evidence from the fields of palaeobotany, phylogeny, and anatomy, but paid particular attention to the geographical distribution of the various growth forms of Dicotyledons and its bearing on the problem of the evolution of herbs. In the eight years that have elapsed since the appearance of our paper, the anatomical aspect of this problem has been taken up in a number of contributions from Professor Jeffrey's laboratory. This series has culminated in two recent papers by Jeffrey and Torrey⁴ in which a vigorous attempt is made to discredit our work. The unfortunate tone of this attack we can afford to ignore, but, in view of the misrepresentation of our position which these and earlier papers contain, and because of the confusion which they have introduced into the discussion, we feel that it is wise to restate our conclusions in the light of all the facts which have been brought forward, and to endeavour to clarify the real points at issue.

¹ Sinnott, E. W., and Bailey, I. W.: Investigations on the Phylogeny of the Angiosperms: No. 4. The Origin and Dispersal of Herbaceous Angiosperms. *Ann. Bot.*, xxviii. 547-600.

² Hallier, H.: Ein zweiter Entwurf des natürlichen (phylogenetischen) Systems der Blütenpflanzen. *Ber. Dent. Bot. Gesellsch.*, xxiii. 85-91, 1905.

³ Eames, A. J.: On the Origin of the Herbaceous Type in the Angiosperms. *Ann. Bot.*, xxv. 215-24, 1911.

⁴ Jeffrey, E. C., and Torrey, R. E.: Physiological and Morphological Correlations in Herbaceous Angiosperms. *Bot. Gaz.*, lxxi. 1-31, 1921. *Ibid.*, Transitional Herbaceous Dicotyledons. *Ann. Bot.*, xxxv. 227-49, 1921.

As a basis for their criticism of our position, Jeffrey and Torrey twice quote from our paper the following paragraph :

'The fact, however, which militates most strongly against the validity of the hypothesis under discussion is that, in practically all many-bundled herbaceous stems, the interfascicular parenchyma is not subtended by tiny leaf-trace bundles, nor is the stem composed of presumably typical alternating large and small bundles, the latter being leaf-traces.'

This they evidently construe to mean a denial by us that the woody tissue confronting the entering leaf-trace may ever become converted into parenchyma, and to refute this supposed conclusion they cite a series of instances, drawn from the immediate region of the node, where such transformation does indeed take place. Upon this basis they further interpret our position as a denial of the presence in herbaceous types of so-called foliar rays, and they maintain that, on the contrary, these foliar rays not only occur in herbs, but are actually an essential feature of the structure of all herbaceous stems.

It is to be emphasized, in this connexion, that in our previous paper we did not deny that a transformation of xylem into parenchyma may occur in the nodal regions of aerial stems. The passage quoted by Jeffrey and Torrey, on the basis of which they attribute such a denial to us, continues as follows :

'On the contrary, all bundles in the aerial stem of a multifasciculate herb tend to be of the same general size, the leaf-traces in the stem usually growing a little smaller as they near their point of departure. At this point, also, the segment of secondary wood which each of them subtends usually grows smaller too, and *may become partially disintegrated into parenchyma.*'

What we *did* deny is that the many-bundled condition existing *throughout* the aerial portion of the stem is due to a conversion into parenchyma of segments of the stem opposite the leaf-traces; and that the interfascicular parenchyma in the long internodal portions of the stem is subtended by a xylem bundle. Both of these statements are amply borne out by the facts. Furthermore, it may be remarked in passing that the term 'foliar ray', in its present sense, has come into use since the publication of our paper. The criticism which has been directed against us on these points, and which forms the basis for the attack upon our position, is, therefore, quite fruitless, and is founded upon an inaccurate statement of our views.

The significant portion of Jeffrey and Torrey's work is their theory of the 'foliar storage ray', and of the part which it has played in the evolution of herbs. Obviously a clear conception of what is meant by 'foliar ray' must be borne in mind if we are to discuss it. In their recent papers, Jeffrey and Torrey describe the foliar ray as a mass of storage parenchyma which has been developed in relation to the entering leaf-trace. It may confront the leaf-trace, flank it, or do both. In the more woody herbs, it

extends for a considerable distance radially, but is short longitudinally. In the more advanced herbs, where the cylinder is thin, the portion of the ray *confronting* the leaf-trace is reduced and disappears, but the *flanking* portions are well developed and extend a considerable distance downwards. In certain cases, woody segments of the stem which are devoid of vessels, or which are provided with more numerous or larger rays than are the adjoining segments, are referred to as foliar rays. In other cases, masses of parenchyma which are formed after the leaves have fallen are similarly designated. Thus, the term foliar ray appears to be a conveniently elastic one.

In the following pages, we shall show that (1) the foliar ray, as thus defined, includes several morphologically distinct structures; (2) many arborescent and fruticose Dicotyledons do have 'foliar storage rays', and have steles which are dissected into discrete woody strands, such as are considered by Jeffrey and Torrey to be characteristic of advanced types of dicotyledonous herbs; (3) many slender herbaceous stems have continuous vascular cylinders and are devoid of 'foliar storage rays'; and (4) Jeffrey and Torrey's work, in ultimate analysis, is actually a confirmation of our contention that the stele of many-bundled herbs is dissected by interfascicular masses of parenchyma which are not subtended by typical xylem elements on their centrad sides.

Before we present evidence in support of these contentions, it is essential to discuss one aspect of our critics' argument upon which they lay much emphasis. They state that 'obviously a clear conception of the differences of anatomical organization between a woody and an herbaceous stem can best be obtained by comparing the nodal regions of nearly related trees and herbs', and in conformity with this attitude they particularly emphasize nodal conditions, citing facts and drawing conclusions chiefly from this portion of the stem. Such a restriction seems to us to be extremely illogical, for any hypothesis which attempts to account for the breaking up of the woody cylinder into separate bundles in the aerial stem of herbs must explain the dissection of the cylinder in the long internodal regions as well as in the comparatively short portion in the vicinity of the node. Consequently, the evidence which we brought forward in our previous paper, and that which we shall present here, is drawn from the aerial stem as a *whole* rather than from any selected region thereof.

That there are several distinct morphological phenomena which are significant in any general discussion of foliar rays, is shown by a comparative study of the aerial stems of numerous representatives of the various orders and families of Dicotyledons. Such an investigation reveals the following facts: (1) The radially disposed sheets of parenchyma, which are commonly known as medullary rays, vary greatly in number, width, height, and distribution in different Dicotyledons. In one group of trees, shrubs, vines, and

herbs, the rays are all narrow and are more or less uniformly distributed. The secondary xylem forms a continuous, unbroken cylinder—except for the foliar gaps at the nodes—regardless of whether the primary elements tend to be aggregated into more or less distinct strands or not (Figs. 1, 3, 5, 11, and 14). In another group of trees, shrubs, vines, and herbs, having sharply defined primary bundles, the gaps between the strands of primary xylem are confronted in the secondary wood by wide sheets of ray parenchyma. Thus, the stele, both in the nodal and in the long internodal portions of the stem, is dissected into a ring of discrete woody segments (Figs. 2, 4, 6, 8, and 9). In the first annual ring these wide rays tend to have a very considerable height, i. e. longitudinal extension (Figs. 19–22). Therefore the leaf-trace segments, which are commonly set off some distance below the points of entrance of the leaf-traces, are flanked on either side by wide sheets of ray parenchyma. At the node these pairs of flanking rays unite with the parenchyma of the foliar gaps (Figs. 21 and 22). (2) In most Dicotyledons there is a tendency for the vessels of the secondary xylem to curve around, and thus to avoid, the entering leaf-traces (Fig. 18). In extreme cases the segment of xylem, confronting the leaf-trace in the stem, may be devoid of vessels for a considerable distance below the node. This phenomenon appears to be closely associated with the conduction of water to the upper levels of the stem, and the vessel-less tissue cannot be regarded morphologically as ray tissue. (3) In many Dicotyledons the rays, in the segments of wood confronting the leaf-traces, tend to be more numerous or wider, particularly in the vicinity of the node (Figs. 7 and 15). (4) In certain trees, shrubs, vines, and herbs, the fibres in the segments of wood confronting the incoming leaf-traces tend to be replaced by vertical parenchyma (Fig. 19).

The last three phenomena may occur in plants which have narrow rays and an unbroken vascular cylinder, as well as in those which have wide, high rays and a ring of discrete woody segments. In stems of the latter type, the substitution of vertical parenchyma for fibres, in the segment of secondary xylem confronting the incoming leaf-trace, bridges the interval between the large flanking rays (Fig. 19). Thus, in tangential, longitudinal sections, the leaf-trace enters the stele through a jacketing mass of heterogeneous parenchyma: ray parenchyma, vertical parenchyma, and parenchyma of the foliar gap (Figs. 13 and 19).

Jeffrey and Torrey interpret the second and third phenomena, outlined above, as stages in the evolution of large storage rays, and actually refer to segments of the cylinder in which they occur as foliar rays. The 'compounding' of a homogeneous mass of storage parenchyma is supposed to occur through the intervention of the fourth phenomenon. Their principal conclusions concerning the evolutionary history of the foliar storage ray are the following:

'3. In the aerial axes of woody herbs a constant and practically never-failing distinction from trees is the formation of large foliar storage rays about the incoming leaf-traces, as they pass through the woody cylinder. 4. In woody herbs the foliar storage rays are well developed in the radial direction, but their vertical extension is slight. 5. In the aerial stems of more slender and less woody dicotyledonous herbs the foliar rays become elongated vertically to compensate for their reduced radial dimension resulting from the thinning down of the woody cylinder. 6. In rays of the type described in 5, the lower part of the radial parenchyma related to the foliar trace is often bifurcated by a tongue of unmodified wood. 7. The vertical elongation of the foliar rays and their subdivision in the manner described in 6 result in the final separation of the originally continuous woody cylinder into a series of separate strands. 8. The final stage of the herbaceous Dicotyledons is a condition in which the cylinder is thinned to such a degree that the radial extension of the foliar rays is virtually eliminated. With this condition is usually associated a great development in length of the portions of the foliar ray flanking the leaf-trace on either side.'

As evidence in favour of conclusion No. 3, Jeffrey and Torrey figure, on the one hand, stems of trees (*Tilia*, *Ulmus*, and *Robinia*) which are devoid of foliar rays and which have continuous vascular cylinders, and on the other hand stems of herbs (*Abutilon*, *Oenothera*, *Boehmeria*, *McIlilotus*, and various Compositae) which have foliar storage rays and which possess more or less conspicuously dissected steles. That the material selected by them is not representative for all trees and herbs, and is, therefore, extremely misleading, is shown by a study of the comparative anatomy of the Dicotyledons as a whole. Trees with 'foliar rays' occur in family after family of the Dicotyledons from the Casuarinaceae to the Compositae. Not only do many trees have the so-called foliar rays, but many of them have the vertically elongated, flanking rays which are considered by Jeffrey and Torrey to be characteristic of an advanced stage of herbaceousness. The arborescent Dilleniaceae, for example, have 'foliar rays' which are of the same type as those figured by Jeffrey and Torrey¹ for *Helianthus annuus* and *Boehmeria nivea*. In these woody types, the entering leaf-traces are entirely enclosed in a jacketing mass of parenchyma: flanking parenchyma, confronting parenchyma, and parenchyma of the foliar gap (Figs. 13 and 19). The confronting parenchyma extends some distance below the node (Fig. 19), and the flanking sheets of storage tissue are projected far down the stem (Fig. 20). Thus, the vascular cylinder is dissected into a series of discrete woody segments which are separated by wide rays (Fig. 10).

It is evident, therefore, that Jeffrey and Torrey's conclusions, that 'in the aerial axes of woody herbs a constant and practically never-failing distinction from trees is the formation of large storage rays about the incoming leaf-traces' and that 'the vertical length of the foliar ray, other things being

¹ Bot. Gaz., lxxi, Pl. II, Fig. 12; Ann. Bot., xxxv, Pl. XII, Figs. 18 and 19; Pl. XIII, Fig. 32.

equal, has a direct relation to the degree of herbaceousness of the axis', are invalidated by many important facts in the comparative anatomy of the Dicotyledons.

Not only have these investigators erred concerning the distribution of so-called foliar storage rays, but they also appear to be mistaken in their interpretation of the origin of these structures. According to their view, the foliar storage rays originate in woody herbs as shallow, radially elongated masses of parenchyma. With increasing herbaceousness these structures become extended downwards and bifurcate, forming sheets of parenchyma which flank the leaf-trace segments in the internodal portions of the stem. Careful study of a wide range of plants, however, indicates that the evolutionary history of the 'foliar ray' has been quite different from this. We have already stated that vertically elongated types of 'foliar storage rays' occur in many arborescent Dicotyledons. That the flanking portions of these masses of parenchyma actually are ordinary multiseriate rays which have fused at the node or have been united by the formation of intervening parenchyma is indicated by a considerable body of facts. In many trees and shrubs, the long flanking portions of the so-called foliar rays are present, but the radially elongated confronting masses of parenchyma are absent. In other words, the pairs of high multiseriate rays which flank the leaf-trace segments do not fuse at the node (Figs. 21 and 22), but merge into the parenchyma of the foliar gaps, forming structures which, in tangential longitudinal sections of the stem, resemble inverted tuning-forks. Various stages in the fusion of such flanking rays, or of their apparent union by the metamorphosis of intervening woody tissue, may occur in different parts of a given individual or in different representatives of a particular genus or family. Furthermore, in multifasciculate herbs, as shown by many of Jeffrey and Torrey's own figures, the long 'foliar storage rays' are not homogeneous bifurcated masses of parenchyma, but consist of two structurally distinct portions, the flanking parenchyma and the confronting parenchyma (Fig. 19). The latter is composed of vertical parenchyma, or of a heterogeneous mass of vertical parenchyma and of fascicular ray parenchyma. It is clearly differentiated from the two sheets of interfascicular ray parenchyma which flank it on either side and which extend downwards into the internode (Fig. 20). Such facts as these indicate very clearly that the flanking portions of the so-called foliar storage rays are not prolongations of originally shallow masses of radially disposed nodal parenchyma, but are a pair of high multiseriate rays whose upper extremities have fused with the independently formed confronting parenchyma.

Among vines and herbs, as among trees and shrubs, there are forms which have narrow rays and essentially continuous woody cylinders (Figs. 3, 5, 11, and 14), as well as forms with high multiseriate rays and steles which are dissected into a series of discrete woody segments (Figs. 4, 6, and 8).

Thus, many herbaceous Centrospermae, Contortae, Tubiflorae, Campanulaceae, &c., with slender aerial stems, are devoid of 'foliar storage rays'. The leaf-traces are not confronted, even at the nodes, by wide sheets of parenchyma (Figs. 14, 16, and 17), nor are they flanked by high multiserial rays. The stele is continuous and unbroken, except for the presence of foliar gaps at the nodes. In certain of these forms (Fig. 14), particularly plants with a typical decussate phyllotaxy, the secondary xylem confronting the leaf-traces may be devoid of vessels for a considerable distance below the node. This phenomenon, however, is not fundamentally a concomitant of the transformation of woody tissue into parenchyma, but is correlated with the conduction of water to the upper levels of the stem.

It is to be emphasized, accordingly, that not only do trees have foliar rays, but that many herbs are devoid of masses of 'foliar storage parenchyma'. Such herbaceous Dicotyledons obviously have not 'developed from arboreal dicotyledonous types by the formation of storage rays about the leaf-traces'.

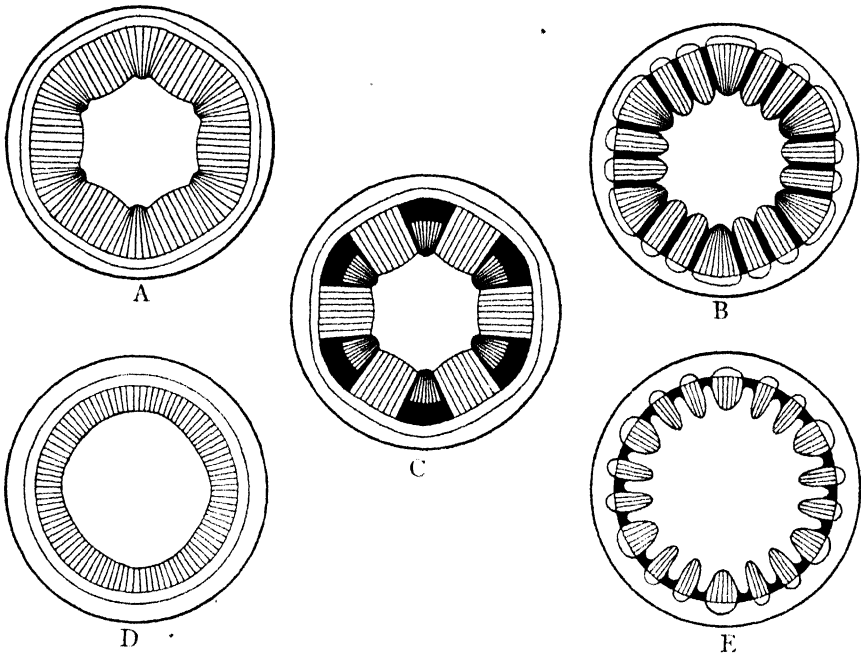
A more detailed study of the comparative anatomy of the Dicotyledons fully justifies our contentions that the aerial stems of herbs are similar to the young stems of their arborescent and fruticose relatives, and that the reduction of secondary growth is the most significant anatomical concomitant of the herbaceous habit. Certain phenomena, such as the widening and lengthening of medullary rays, the disappearance of secondary vessels in certain segments of the woody cylinder, an increase of parenchyma in the nodal regions, &c., may be accentuated in certain groups of herbs, vines, and lianas, but they are not essential concomitants of herbaceousness, and are often present in arborescent forms. Furthermore, Jeffrey and Torrey admit the essential correctness of our conclusion that the stele of many-bundled herbs is dissected by interfascicular masses of parenchyma which *are not subtended by primary wood on their centrad sides*. They state:

'The foliar ray surrounding and subtending the leaf-trace is characteristic of the less advanced dicotyledonous herbs and of the lower, more woody regions of those higher in the herbaceous sequence. This type of ray gives place by the later thinning of the woody cylinder to one in which the storage tissue is confined to the flanks of the traces. The long vertical extension of these flanking rays results in the division of the originally continuous woody cylinder of the ancestral Dicotyledons into a circle of separate strands, the fibro-vascular bundles.'

The last sentence of this quotation is in fact an admission of the correctness of the view which we expressed in our previous paper, that the flanking rather than the confronting parenchyma is responsible for the breaking up of the woody cylinder into separate bundles. It should be noted in this connexion that, according to the present position of Jeffrey and Torrey, the confronting portion of the foliar ray, emphasized by them

as of so much importance, is really but a temporary phase in the evolution of the herbaceous stem, since it is stated to be absent in the woody prototypes, to be present in transitional herbs, and to disappear again as the herbaceous structure reaches its fullest development.

The essential difference between our position and that of Jeffrey and Torrey as to the evolution of the herbaceous stem may be concisely shown by the following series of diagrams. Our critics derive the herbaceous type from such a woody stem as is represented in A, where wide rays, flanking



A. Transverse section of one-year-old stem of arborescent or fruticose Dicotyledon, which is devoid of wide, high multiseriate rays. B. Transverse section of one-year-old stem of arborescent or fruticose Dicotyledon whose woody cylinder is dissected into separate strands by wide, high multiseriate rays. C. Transverse section of stem of woody or 'transitional' herb showing 'confronting' and 'flanking' parenchyma—the condition which is emphasized by Jeffrey and Torrey in their theory of the origin of the herbaceous type. D. Transverse section of slender, herbaceous stem which is devoid of 'foliar storage rays'. E. Transverse section of slender, herbaceous stem whose stele is dissected into a series of discrete woody strands.

the leaf-traces, are absent. From this ancestor arises the transitional herb, C, possessing 'foliar rays', a stage particularly emphasized by Jeffrey and Torrey. From this, in turn, through the disappearance of the confronting portion of the foliar ray is derived the typical, many-bundled herbaceous stem of advanced type, E. In this sequence conditions at the node are particularly emphasized.

According to the view of the writers, based on a study of the whole aerial stem, the condition in E may be derived directly from such a woody ancestor as shown in B. In the latter type well-marked flanking rays are

present which in the first annual ring break up the woody cylinder into separate strands. The development of a typical many-bundled herbaceous stem from such a condition as this is merely a matter of a progressive thinning of the vascular cylinder. In a similar manner, herbaceous stems with unbroken cylinders, D, may be derived directly from A by a general reduction in cambial activity. The explanation proposed by us—aside from its greater directness and the fact that it does not necessitate the development of a new structure which is ultimately lost again before the evolutionary series has reached its final stages—possesses the advantage of not being invalidated by the occurrence of ‘foliar storage rays’ in many arborescent Dicotyledons, or by their absence in certain groups of herbs.

SUMMARY.

1. Recent criticism of our work on the comparative anatomy of woody and of herbaceous Dicotyledons is based upon an inaccurate statement of our views and has therefore confused the issues involved.

2. Our critics attack the conclusions that a progressive thinning of the woody ring is the chief anatomical concomitant of the change from a woody to an herbaceous habit, and that the woody ring of many-bundled herbs is dissected by high, multiseriate rays which are not subtended by woody elements on their centrad sides.

3. They assert that a new structural feature, the ‘foliar storage ray’, has made its appearance in the evolution of herbs, and that herbaceous stems, therefore, cannot be regarded as essentially similar in construction to the young stems of their woody ancestors.

4. Evidence is here presented that (1) the ‘foliar storage rays’ of our critics include several morphologically distinct structures; (2) many trees and shrubs among Dicotyledons do possess ‘foliar rays’, and therefore display, in their first annual rings, dissected steles precisely comparable to those occurring in herbaceous types; and (3) many herbaceous stems, on the contrary, have essentially continuous vascular cylinders and thus do not possess ‘foliar storage rays’.

5. The hypothesis of our critics that trees and shrubs gave rise to ‘woody’ or transitional herbs through the development of well-marked ‘foliar storage rays’, and that woody herbs gave rise, in turn, to the still more herbaceous types by a loss of the confronting portion of the ‘foliar ray’ and a downward extension of its flanking portions, is open to criticism, since it does not explain the origin of herbs with continuous cylinders, and since it involves the evolution and the subsequent loss of a transitional structure, when the facts can more easily and simply be explained without such an assumption. Indeed, in their discussion of the more herbaceous types, the correctness of our major contention is essentially admitted by our critics.

6. The evidence therefore seems still to support our view that the herbaceous stem in general is essentially similar to the first year's growth of its woody prototype, differing mainly in the possession of a relatively thinner woody ring. Where the rays are small, the vascular cylinder in both woody plants and herbs is practically continuous; where wide and high rays occur, they break up the stele into separate strands in both types.

DESCRIPTION OF PLATES XVIII AND XIX.

Illustrating Messrs. Sinnott and Bailey's paper on the Significance of the 'Foliar Ray' in the Evolution of Herbaceous Angiosperms.

PLATE XVIII.

Fig. 1. *Liquidambar*, Tree. Transverse section of stem, showing narrow rays and unbroken fibro-vascular cylinder. $\times 15$.

Fig. 2. *Platanus*, Tree. Transverse section of stem, showing wide rays and many-bundled type of stele. $\times 27$.

Fig. 3. *Lonicera*, Vine. Transverse section of stem, showing narrow rays and unbroken fibro-vascular cylinder. $\times 32$.

Fig. 4. *Clematis*, Vine. Transverse section of stem, showing wide rays and many-bundled type of stele. $\times 14$.

Fig. 5. *Digitalis*, Herb. Transverse section of annual stem, showing slender unbroken fibro-vascular cylinder, which consists almost entirely of primary elements. $\times 11$.

Fig. 6. *Artemisia*, Herb. Transverse section of stem with slight cambial activity, showing many-bundled type of stele. $\times 24$.

Fig. 7. *Drumys*, Tree. Transverse section of node, showing increase in the number and size of the rays in the leaf-trace segment. $\times 55$.

Fig. 8. *Eupatorium*, Herb. Transverse section of woody stem, showing wide rays and many-bundled type of stele. $\times 33$.

Fig. 9. *Plantanus*, Tree. Portion of Fig. 2 more highly magnified. $\times 55$.

Fig. 10. *Dillenia*, Tree. Transverse section of internode, showing leaf-trace and wide (multi-seriate) flanking rays. $\times 60$.

Fig. 11. *Digitalis*, Herb. Portion of Fig. 5 more highly magnified, showing xylem, phloem, and jacketing layer of sclerenchyma. $\times 33$.

Fig. 12. *Dillenia*, Tree. Transverse section cut just below the node, showing small lateral leaf-trace and fusion of flanking rays. $\times 60$.

PLATE XIX.

Fig. 13. *Dillenia*, Tree. Transverse section of node, showing large lateral leaf-trace confronted by 'foliar storage tissue'. $\times 50$.

Fig. 14. *Campanula*, Herb. Transverse section of node, showing absence of 'foliar storage tissue'. The entering leaf-trace is confronted and flanked by prosenchymatous tissue. $\times 30$.

Fig. 15. *Cercidiphyllum*, Tree. Transverse section of node, showing decrease of vessels and increase of rays in vicinity of entering leaf-trace. $\times 38$.

Fig. 16. *Campanula*, Herb. Tangential longitudinal section of slender herbaceous stem, showing entering leaf-trace and parenchyma of foliar gap. The confronting and flanking tissue is prosenchymatous and 'foliar storage tissue' is absent. $\times 20$.

Fig. 17. *Chelone*, Herb. Tangential longitudinal section of slender herbaceous stem, showing entering leaf-trace and parenchyma of foliar gap. The confronting and flanking tissue is prosenchymatous and 'foliar storage tissue' is absent. $\times 50$.

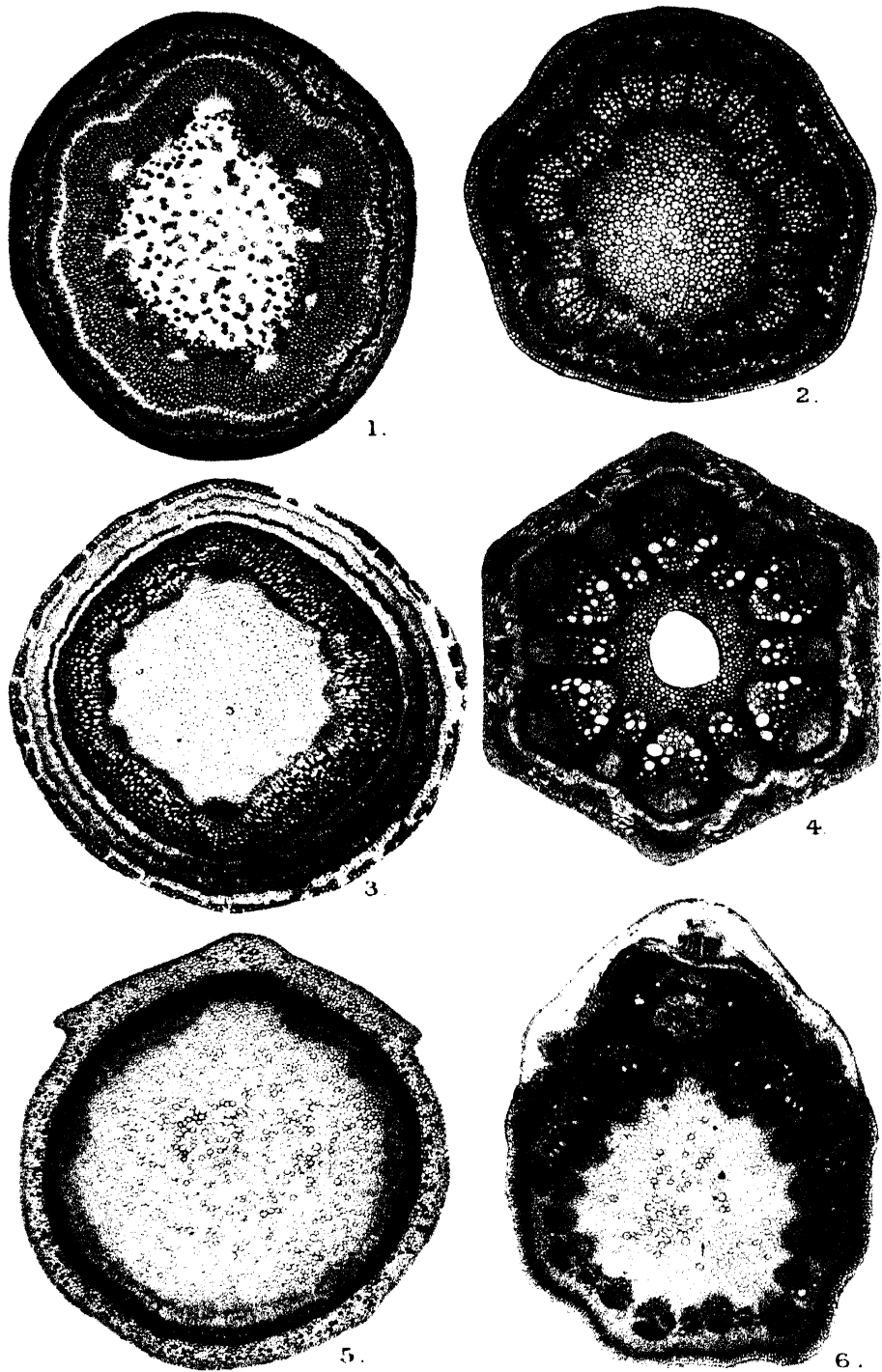
Fig. 18. *Asclepias*, Herb. Tangential longitudinal section of node, showing entering leaf-trace and parenchyma of foliar gap. The vessels of the secondary xylem are curving around, and thus avoiding the leaf-trace. $\times 21$.

Fig. 19. *Dillenia*, Tree. Tangential longitudinal section of stem, showing entering leaf-trace and so-called foliar ray. The wide, very high 'flanking' rays are united for a considerable distance below the node by 'confronting' parenchyma. The cells of the latter tissue are vertically elongated. $\times 42$.

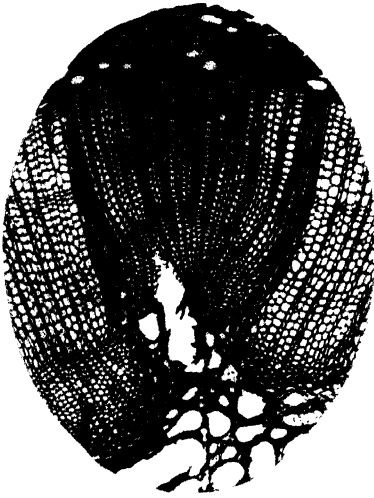
Fig. 20. *Same*. At a lower level of the stem, showing flanking rays and dying out of confronting parenchyma. $\times 42$.

Fig. 21. *Liiodendron*, Tree. Tangential longitudinal section of stem, showing fusion of large, high flanking rays with the parenchyma of the foliar gap. $\times 31$.

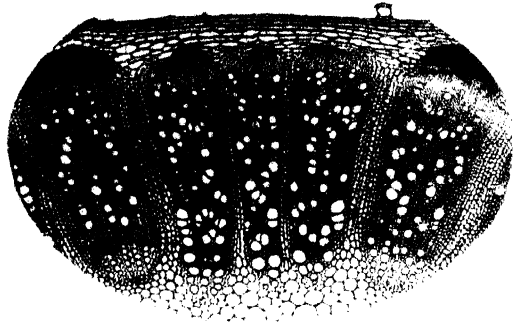
Fig. 22. *Acanthopanax*, Tree. Tangential longitudinal section of stem, showing fusion of high flanking rays with parenchyma of foliar gap. $\times 33$.



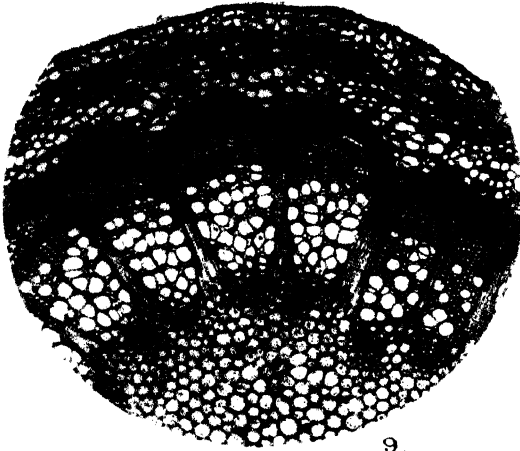
SINNOTT & BAILEY—FOLIAR RAY.



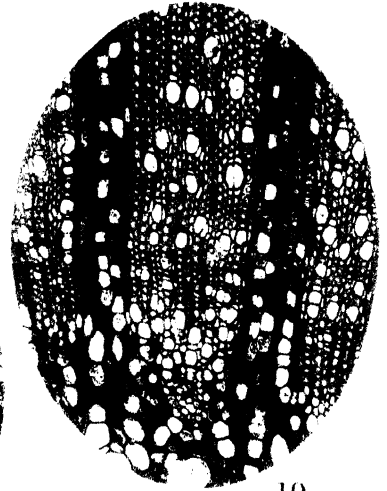
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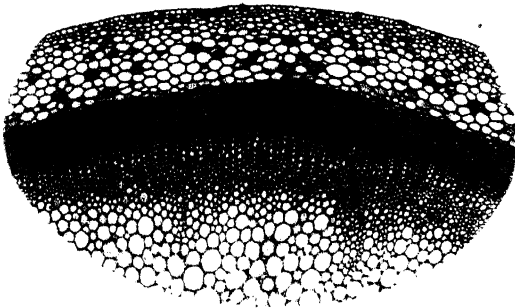
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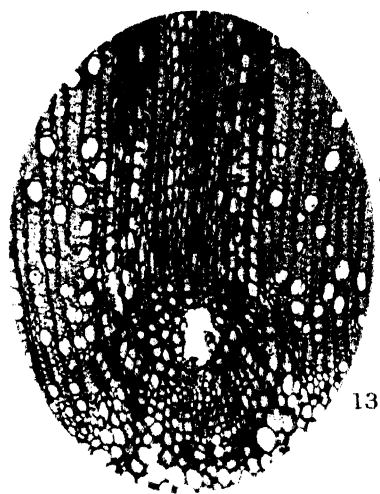


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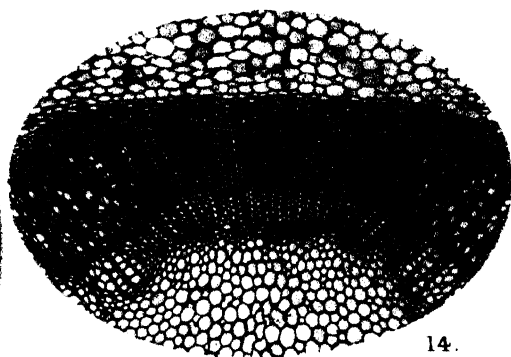


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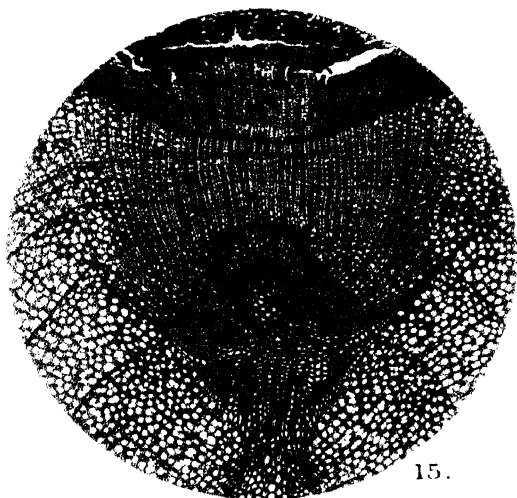
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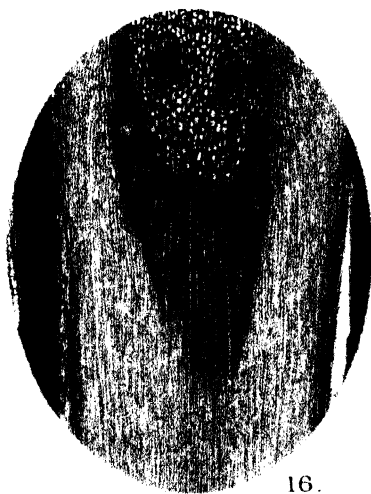
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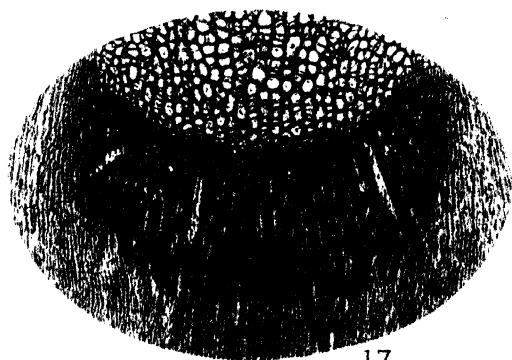
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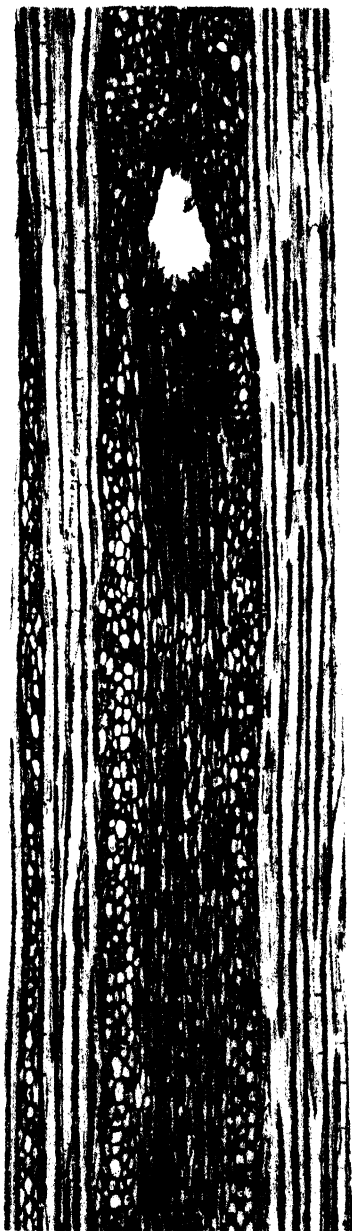
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22.



20.

Spermatogenesis in *Asterella hemisphaerica*, Beauv.

BY

WILLIAM LOGAN WOODBURN,¹

Late Associate Professor of Botany in Northwestern University, Evanston, Ill.

With Plate XX.

THE material for this paper was collected several years ago, about five miles south-east of Bloomington, Indiana ; and it was killed in chromic-osmic-acetic acid. Upon working up the material the writer found that certain stages were not abundant, especially the last divisions of the spermatogenous tissue and the early stages in the development of the androcyte, or the protoplast which functions as a sperm. However, since so many definite stages in spermatogenesis and many cytological phenomena are clearly demonstrated, it was thought best briefly to summarize the data at present available.

Within recent years, investigators of the Hepaticae (especially in spermatogenesis) have reported cytological phenomena so varied that further information seems desirable. In an article by the writer on *Mnium*, in 1915, certain of these reports prior to that date were very briefly summarized². Other papers by various investigators have appeared since. Without repeating here in detail, we may say that the evidence indicates considerable variation, throughout the group consisting of the Hepaticae and the Musci, in two rather important respects: first, as to the origin, nature, and general prevalence of a centrosome-like structure, which is apparently the primordium of the blepharoplast ; second, as to the presence and nature of certain accessory bodies found in the cytoplasm of spermatogenous cells.

The student of botany to-day dare not draw dogmatic conclusions from isolated results of investigation. We are too well aware of the fact that extreme variability and plasticity constitute most prominent characteristics of the plant cell. Yet it seems reasonable to expect that we may find underlying this variability the expression of certain laws which are constant

¹ Owing to the death of the author this paper has been arranged for publication by Professor Atwell, of Northwestern University, and Professor Mottier, of Indiana University.

² Ann. Bot., xxv. 299-313, 1911 ; *ibid.*, xxvii. 93-100, 1913 ; *ibid.*, xxix. 441-56, 1915.

in operation, and the regular appearance of fundamentally significant structures which indicate natural relationships. The visible architecture of the cell is constantly changing. A nuclear or cytoplasmic structure of a certain pattern may be organized, pass through a series of changes, and then apparently be resolved again into less highly differentiated protoplasm. While throughout the majority of the plant groups we recognize the constant separation of the protoplast into nucleus and cytoplasm, the majority of certain forms (for example, the Schizophyta) fail to exhibit this differentiation. There is also a time in the life-history of a Bryophyte when an intimate union, if not an intermingling, of nucleus and cytoplasm seems to exist. The protoplast which forms the male gamete exhibits at early stages the usual sharp division into nucleus and cytoplasm. As the sperm reaches maturity, this sharp distinction between nucleus and cytoplasm becomes less defined. Perhaps in no other case is the morphological unity of the protoplast as a whole more clearly demonstrated. Within this unit there is a distinct organization of the elements, which are constantly mobile, and which become grouped according to definite laws, with the consequent appearance of visible protoplasmic structures. The individual protoplast as a unit of organization is clearly apparent. The writer does not wish to minimize the value of minute structural details, but merely to emphasize at the same time the power of the protoplast to develop along definite morphological and physiological lines with considerable plasticity of structure. The following data from *Asterella* confirm largely the writer's previous conclusions in regard to other Bryophytes.

THE PROPHASE IN ANTHERIDIAL TISSUE.

The fixed and stained cytoplasm of an antheridial cell in a resting condition contains very finely divided granules (Pl. XX, Figs. 1 and 2). These granules are not evenly distributed, but are aggregated irregularly into flocculent masses. These masses are of varying density and structure. One may find more or less uniform gradation from open, granular cytoplasm, through areas of more closely grouped granules, to larger, deeply-stained lumps. That these larger granules appear in any definite number or pattern, the writer finds no evidence.

The nucleus contains an irregular network of relatively large granules with one larger, centrally-placed, spherical body which resembles a nucleolus (Figs. 1 and 2). A faintly-stained or quite clear region may completely surround this central body. In or around this body, or nucleolus, the chromatin gradually collects during the prophase of division (Figs. 1, 2, and 3). As a result, this central mass gradually loses its regularly spherical form and becomes a larger group of irregular chromatin lumps, which presents somewhat the appearance of synapsis in spore-mother-cells (Fig. 4). Occasionally (Fig. 4) one lump stains like a nucleolus as compared with the

larger masses of chromatin. In most cases, however, the original nucleolus seems to enlarge through a concentration of the chromatin. As the nucleolus continues to enlarge and becomes more loosely arranged (Figs. 4 to 7), the chromosomes assume definite form. The number of chromosomes seems to be not less than eight. A continuous and distinct spireme was not observed.

THE FORMATION OF THE SPINDLE.

The first indication of the development of the spindle which was observed was the formation of cap-like areas of cytoplasm at opposite sides of the nucleus (Figs. 8 and 9). These caps may be closely applied to the nuclear membrane (Fig. 8), or only slightly removed from it (Fig. 9). These caps may remain for some time slightly beyond the extremities of the poles of the spindle. Soon after their appearance the nucleus becomes drawn out along the axis in which these cytoplasmic caps lie (Fig. 5). From this material, very delicate intranuclear spindle fibres soon become organized (Figs. 5, 10, and 11). As the spindle reaches maturity, remnants of the polar cap may occasionally be seen (Fig. 10). Occasionally a small granule may occupy the sharp-pointed pole of the spindle (Fig. 10). However, the poles may be sharp-pointed without the presence of either polar caps or granules (Fig. 11). Or, again, the poles may be broad and truncated (Fig. 12). Sometimes the spindle may be multipolar (Figs. 12 and 13) with more or less conspicuous granules occurring where the fibres converge. A careful study of Figs. 8, 9, 11, 12, and 13 would lead to the conclusion that, while polar granules occur with irregularity, no bodies are evident which partake clearly of the nature of centrosomes. Similar granules may occur with irregularity in number and position throughout other portions of the cytoplasm.

A careful examination of the stages represented in Figs. 1, 5, 8, and 13 has convinced the writer that, in *Asterella*, polar caps and granular bodies arise *de novo* as the nucleus enters upon division. There seems to be no basis for assigning to them morphological rank more than to the fibres of the spindle. They seem to be temporary structures of protoplasmic development, as are the spindle fibres. The caps no doubt actually become a part of the spindle. There is no evidence that the polar caps or granules (Figs. 5, 8, 9, and 10) are permanently organized structures. Their appearance and behaviour suggest rather a visible structure which is built up temporarily and which performs definite functions, and then is resolved into less differentiated cytoplasm. This conception, supported by cytological evidence, seems to the writer to throw some light on the questions suggested above.

The reaction of the delicate protoplasmic structures to killing fluids is also significant, as a comparison of different cells in different parts of the antheridium will show.

TELOPHASE.

Various stages of telophase were carefully examined, but no body was discovered which might with certainty be termed a centrosome or a blepharoplast (Figs. 14-17).

FORMATION OF THE BLEPHAROPLAST AND DEVELOPMENT OF THE SPERM.

The blepharoplast was first seen as a densely-staining body, somewhat elongated, lying close to the boundary of the protoplast which later functions as a sperm.

Two sperms are formed from one of the cubical cells (androcyte mother-cells) of the antheridium. The last spindle does not occur obliquely with the same regularity as in *Marchantia* or *Conocephalum*. All of the cytoplasm of the androcyte mother-cell is not used in the formation of the two sperms. Each of Figs. 19 to 21 shows one of the pair of sperms found in each of three mother-cells. A definite membrane develops some distance away from the nucleus, but does not enclose all of the peripheral cytoplasm. The blepharoplast first makes its appearance upon the inner surface of this membrane, as a deeply-staining lump or short band of cytoplasm (Figs. 19 and 20). The cytoplasm becomes more dense just within the enclosing membrane, leaving the space around the nucleus somewhat vacuolate. Very densely-staining material often collects on the inner surface of this membrane (Fig. 21). It is along this course that the blepharoplast develops. The material which produces the blepharoplasts collects in a rather lumpy granular band. The writer has earlier emphasized the possible close association or intermingling of nuclear and cytoplasmic material during the metamorphosis of the androcyte, and that in certain cases the nucleus and blepharoplast become quite indistinguishable. There is here additional evidence of the outward diffusion of material from the nucleus. In some cases it consists of finely divided or scarcely stained material; in others of larger lumps of coagulated substance. Careful observations were made to determine the effect of the killing fluid upon the structure of the protoplasm. At this stage, cells near the periphery of the antheridium, where the killing fluid had penetrated rapidly, appear as in Figs. 19 and 20; while those into which the fluid had filtered more slowly frequently showed the protoplasm much coagulated, as in Figs. 17 and 18.

Other writers have described the extrusion of material from the nucleus into the cytoplasm during this period.

[Figs. 22 to 27 indicate the remaining steps in the development of the sperm. Manuscript pertaining to this part of spermatogenesis was not found.]

SUMMARY.

The last division in the spermatogenous cells may be oblique, as in *Marchantia*, but it is not always so.

The number of chromosomes is eight.

Prior to the formation of the spindle, small, dense cytoplasmic caps appear on opposite sides of the nucleus near the positions which will be occupied by the poles of the future spindle. These caps arise *de novo*; they are neither permanent cell-structures nor are they centrosomes.

The blepharoplast arises near the plasma membrane as a newly-formed structure.

The most completely developed sperm shown consists of a curved, club-shaped part, the nucleus, tapering to a slender point which is continued by the thread-like blepharoplast bearing two cilia at its anterior end.

EXPLANATION OF PLATE XX.

Illustrating Professor W. L. Woodburn's paper on Spermatogenesis in
Asterella hemisphaerica, Beauv.

Figs. 1 to 17 pertain to the last nuclear and cell division in the spermatogenous cells (androcyte mother-cells) in the antheridium.

Fig. 1. Cell with nucleus in resting condition.

Fig. 2. The chromatin has become more coarsely granular. A large nucleolus lies in a colourless cavity.

Fig. 3. The chromatin shows a tendency to accumulate about the nucleolus.

Fig. 4. The entire nuclear content has contracted near the centre of the nucleus.

Fig. 5. The nucleus has become elongated.

Figs. 6 and 7. The formation of the chromosomes.

Figs. 8 to 13. The formation of the spindle. In Figs. 8 and 9 densely staining caps of cytoplasm appear on opposite sides of the nucleus. In Fig. 10 the caps have almost disappeared. Fig. 11 represents a pointed spindle, while in Figs. 12 and 13 the poles are broadly truncated.

Figs. 14 to 17. Telophases.

Figs. 18 to 27. Transformation of the sperm initial into the mature sperm.

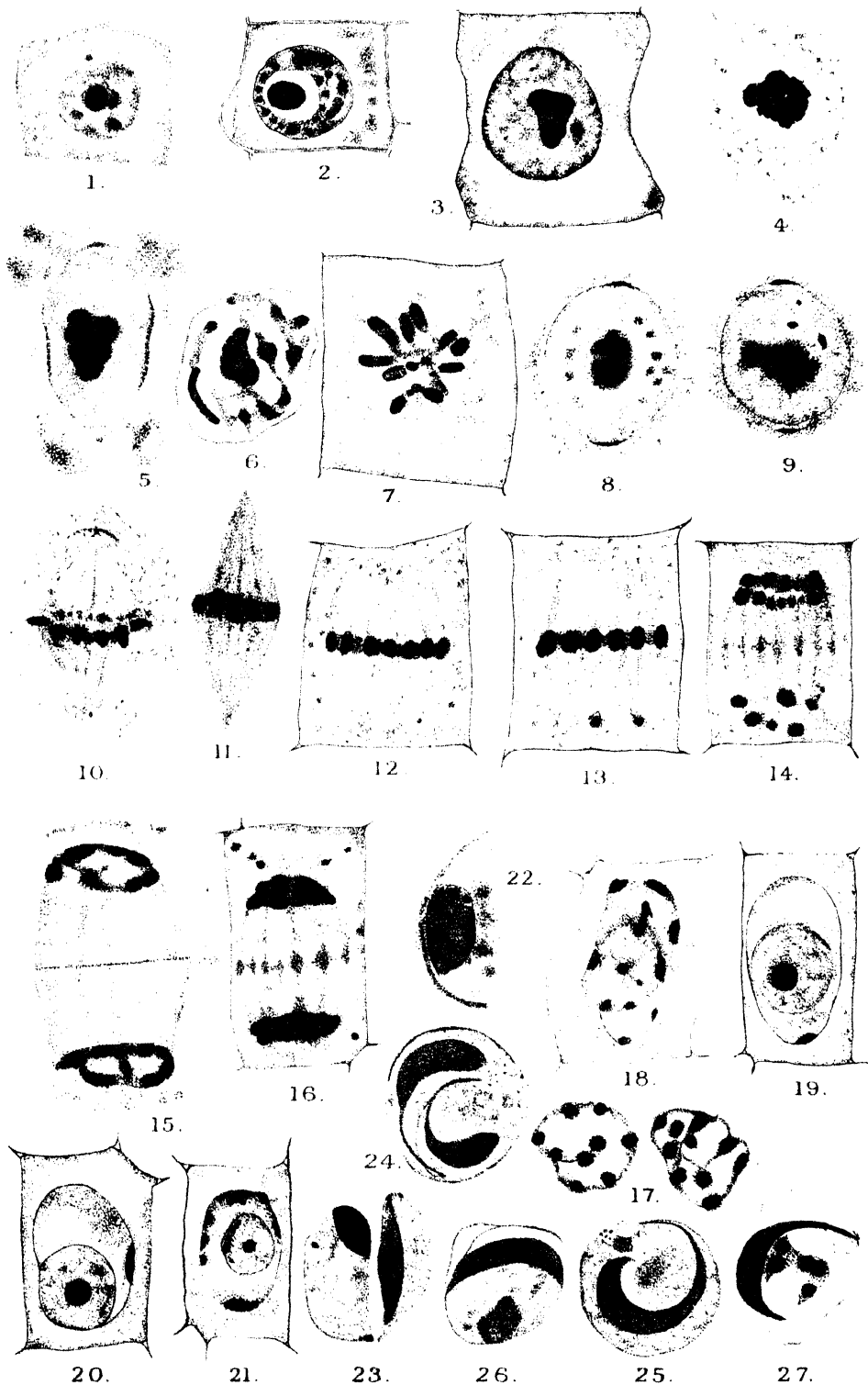
Fig. 19. The plasmic contents have rounded up. A dense body, probably the primordium of the blepharoplast, is seen near the plasma membrane. Finely granular material is indicated between the cell-wall and the rounded protoplast.

Figs. 20 and 21. Later stages.

Figs. 22 and 23. The blepharoplast is now elongated and thread-like; the nucleus is oval or elliptical.

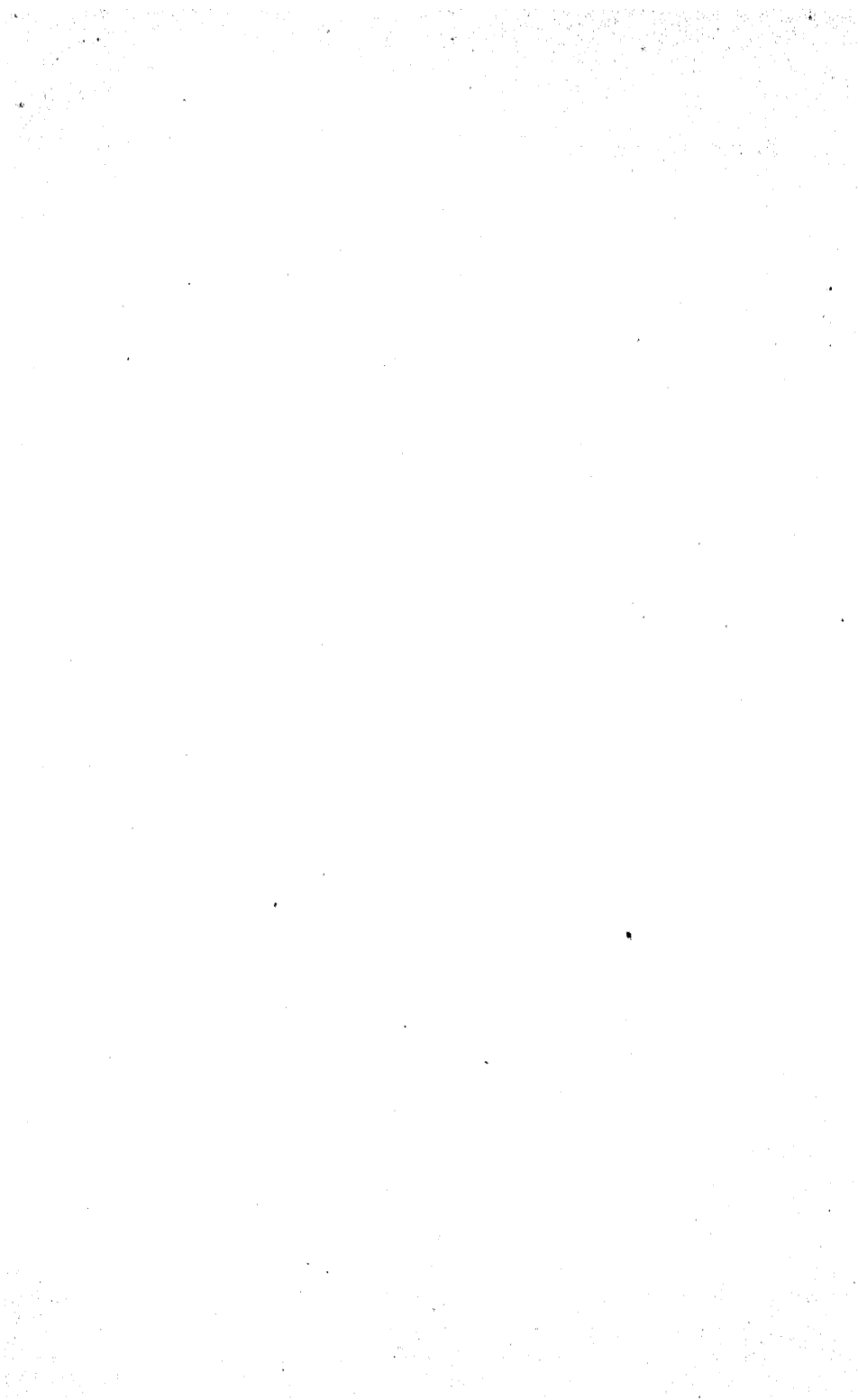
Figs. 24 and 25. Two young sperms of a single androcyte.

Figs. 26 and 27. The cilia are present.



Woodburn ad nat. del.

Huth lith et imp.



Some Types of Endolithic Limestone Lichens.

BY

E. J. FRY, M.Sc.,

Assistant Lecturer in Botany, University College of Wales, Aberystwyth.

With Plate XXI and nine Figures in the Text.

INTRODUCTION.

AN investigation of the pioneer cryptogamic vegetation of the Carboniferous crystalline limestones of the Great Orme and in S.E. Anglesey was begun in 1918. The present paper deals with the study of several of the more common endolithic lichen thalli found on those outcrops.

Up to the present time little work on embedded lichens has been published, and it was thought that, since new and better methods of sectioning the endolithic thalli had been found, it might be advisable to make a detailed study of certain forms which Bachmann (1) did not describe. It is to Bachmann that we owe our present knowledge of the anatomy of such plants.

GENERAL NOTES.

Investigation of the outcrops under discussion shows there is scarcely a square inch not occupied by lichen thalli of some kind, and of these by far the most numerous, both in species and individuals, are the endolithic forms. The majority of these can be detected with the naked eye, since their fruiting organs at maturity appear at the surface, and frequently the endolithic lichens give a tinge of colour to the surface of the rock. The presence of other forms—which fruit but rarely and give very little, if any, colour to the limestone—is betrayed only by the scratching of the rock surface, which gives a bright green or orange ‘streak’ according to whether the algal constituent of the embedded lichen belongs to the Chlorophyceae or the Trentepohliaceae. In some species, e.g. *Verrucaria calciseda*, the boundary of the thallus is clearly marked by a black line. In this region the limiting lichen hyphae are dark coloured and in some cases are raised just above the level of the rock.

Over these embedded thalli creep the epilithic forms such as *Xanthoria parietina*, *Lecanora murorum*, *L. cirrochroum*, and species of *Physcia*, of which only the rhizoids penetrate the limestone already partly broken up by the

- action of the endolithic thalli. Apart* from such epilithic types there are a few whose thalli are partly epilithic and partly endolithic, e.g. *Aspicilia calcarea*, the endolithic part only of which has been described by Bachmann (loc. cit.).

METHOD OF PREPARATION OF MATERIAL.

Bachmann prepared and examined slides of the rock with the lichen *in situ*. To examine the thallus in greater detail he removed the limestone by treating the rock section with hydrochloric acid or Perényi's fluid; but before this could be done all trace of the Canada balsam, used in fixing the rock section to the glass slide for the rubbing-down process in the preparation of rock sections, had to be removed by special methods. It was necessary that this process of decalcification should be carried out on the slide, since the thallus sections were too delicate and of too loose a texture to allow of the usual manipulation. The disadvantages of such a method are obvious. In the first place, rock sections, thin enough to show sufficient detail of the hyphae, cannot be prepared without loss of a certain number—perhaps the majority—of the gonidia, which come out fairly easily in the rubbing-down process. Secondly, in the decalcification of sections, a number of the short portions of hyphae and algae readily break away from such a loose structure, so that a perfect section can rarely be obtained. In the present investigation, rock sections having proved unsatisfactory, the following method was adopted for the preparation of sections of endolithic thalli. From the surface of fairly pure crystalline limestone bearing the endolithic lichens, fragments of about 1 cm.—1.5 cm. thickness were chiselled. These were then immersed in hydrochloric acid. In order that the effervescence might be very slow the acid was made very dilute. This precaution allowed the true structure to be preserved, which might have been destroyed by too violent a reaction. The process in a number of cases has been observed under the microscope. Effervescence was less rapid from the upper surface. This became woolly in appearance as the limestone was dissolved, and the degree of coarseness of the texture varied with the different lichens. At the end of three or four hours all the calcium carbonate was removed. To the naked eye the thallus, freed of the rock, appeared as a thin rind, from the underside of which spread out a meshwork of very fine, soft hyphal threads, the length, number, and colour of which varied with the different species. When the thallus was touched an impression was given as of a mass of sodden silken threads. This mass collapsed on removal from the liquid. The whole was washed gently in running water for twenty-four hours, then fixed in chrom-acetic, washed, dehydrated, embedded, and serial sections cut in the usual way. It should be mentioned that before washing free of the acid the underside of the material was examined under the microscope for insoluble impurities. These, when present, were removed, care being

*I. Wholly Endolithic Types. A.**a. Verrucaria calciseda, D. C.*

Under the microscope the surface of the limestone immediately above *Verrucaria calciseda* appears white and rather granular, exhibiting very numerous black spots and empty pits. The spots are of two kinds. The larger circular patches are the carbonaceous lids of the perithecia, while the smaller dots are the dark external ends of hyphae of the cortical zone, or, as is often the case in these endolithic forms, they are small colonies of minute blue-green algae, which adhere to the surface. The pits are the positions of the old perithecia.

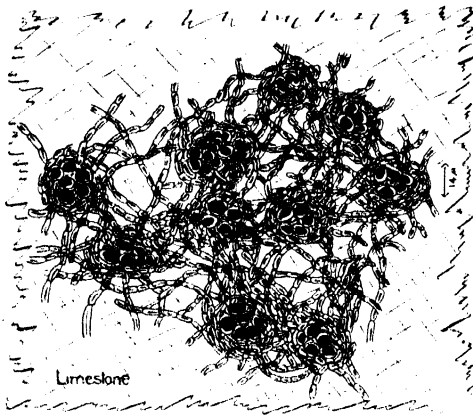
After decalcification the thallus is very thin, soft, and delicate, the upper surface having a fine woolly appearance. Both the upper and lower surfaces are white, as are also the short rhizoids. In a vertical section (Pl. XXI, Fig. 1) can be seen the three zones: cortical, gonidial, and rhizoidal. From the cortex to the limits of the rhizoids measures 670 μ . The whole thallus, except for the short dark hyphal tips, is embedded within the rock.

Cortical zone. The cortex has an average thickness of 28 μ . Although it appears fairly continuous the hyphae are not evenly distributed. In certain regions, i. e. just above the main groups of gonidia, the hyphae are shorter and more closely packed, forming a dense tissue. Linking up these clumps one with another are many branching hyphae which are composed of the short cells or more elongated ones. Towards the surface of the rock the cortical hyphae tend to be of a greyish brown colour. Those that are actually at the surface are a deep brown or black (Pl. XXI, Fig. 1, a).

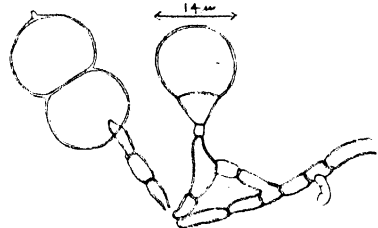
Gonidial zone. The average width of the gonidial zone is about 75 μ . In a longitudinal section the bright green gonidia are arranged in more or less regular, elongated groups. These branch in one or more directions. The long axis of the gonidial group usually is at right angles to the surface of the rock, though frequently it may run almost parallel with it (Pl. XXI, Fig. 1). The gonidia are wound round by innumerable hyphal threads (Text-fig. 1), which, besides enclosing the whole group in a kind of envelope, push their way between the individual gonidia. These hyphae are continuous with the hyphal tissue immediately above the group. The gonidial groups with their hyphal caps are situated in hollows in the limestone, and it is from these that the gonidia are so easily lost in the rubbing process in the preparation of the rock sections by Bachmann's method. From the common envelope of short hyphae with dense contents there are given off numerous branches which connect up the neighbouring groups of algal cells (Text-fig. 1). Other branches pass in a downward direction, branch freely, and anastomose while still in the region of the gonidial zone.

Rhizoidal zone. The hyphae which are deepest in the rock are narrower

and less regular in shape than those nearer the surface. The contents of a cell in the lower rhizoid region are small in comparison with those of a hypha of the gonidial zone, but the nucleus is very plainly marked. In the upper rhizoidal zone the hyphae resemble very closely those in the neighbourhood of the gonidial groups. Commonly the terminal cell or cells of a branch or filament swell up into a spherical body or bodies which often reach a diameter of 14μ or more (Text-fig. 2). These are usually thin walled, but frequently become thicker and dark coloured, particularly in the marginal regions of *Verrucaria calciseda*. These spherical or inflated hyphae are full of a highly refractive greenish oil. Neither the function nor the formation of this substance is properly understood, but various suggestions



TEXT-FIG. 1. *Verrucaria calciseda* (tangential section 30μ in limestone), showing gonidial groups encircled by hyphae, and hyphae crossing from group to group.

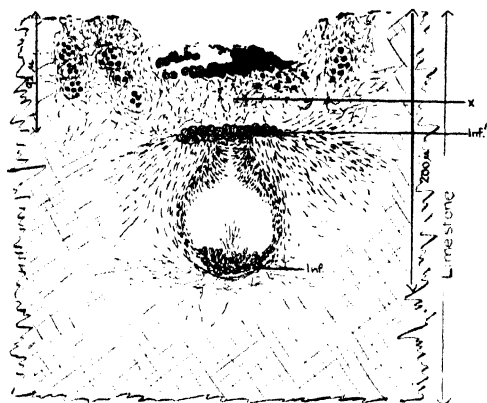


TEXT-FIG. 2. *Verrucaria calciseda*. Simple inflations of hyphae.

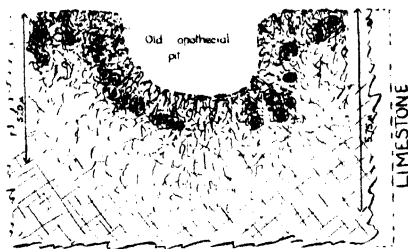
have been made which will be referred to in the general discussion. From numerous cases examined in other forms it seems that the oil accumulates and the wall enlarges or stretches to keep pace with the accumulation. The inflated hyphae which are present immediately below the gonidial zone are found commonly all through the rhizoidal region. It has been mentioned that at the margin of the thallus there is a black line. In longitudinal section this shows as a mass of dark-coloured, slightly enlarged hyphal cells, just above the level of the limestone. Immediately below this in the rock the decomposing remains of the neighbouring thallus are seen in the meshes of the dark marginal hyphae (Pl. XXI, Fig. 2). The presence of this dark mass at the surface is not constant. In other places the hyphae do not come above the level of the rock, but remain more loosely packed in the limestone. They are dark coloured, thick walled, and bear numerous terminal inflations. Often they are found in the rock ahead of

the thallus on the surface, penetrating the cortical gonidial and rhizoidal regions of the neighbouring lichen.

The perithecium. The whole perithecium, except for the lid or cap, is embedded in a deep pit in the rock. This pit usually reaches down to two and a half to three times the depth of the gonidial zone (Pl. XXI, Fig. 1, and Text-fig. 3). The fruiting body figured in the plate is not mature; asci are present, but ascospores have not yet been formed. Paraphyses, as is usual in this species, have decomposed and formed mucilage, which is indicated near the base of the neck. Lining the neck are numerous paraphyses. The lid already shows the position of the pore. Even at immature stages the fruiting body never completely fills the hollow. The origin of the perithecium is within the limestone, and at an early stage the



TEXT-FIG. 3. *Verrucaria calciseda*. Young apothecium (longitudinal section). *Inf.* = inflated hyphae; *Inf'*. = inflated hyphae forming lid; *x* = disorganizing part of thallus and dissolving limestone in process of dissolution.

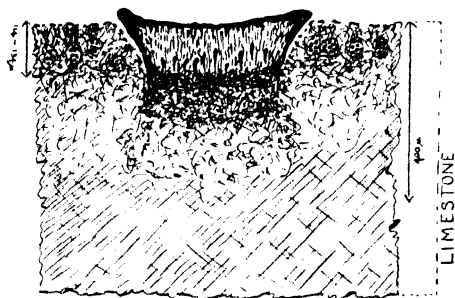


TEXT-FIG. 4. *Lecidea immersa*. Old apothecial pit being recolonized by gonidia and hyphae.

black lid is found to be situated immediately over the developing perithecium, but completely inside the thallus (Text-fig. 3). In the earliest stages this lid is seen to be made up of thin-walled, inflated cells which at a later period turn black and become thick walled, and can scarcely be distinguished (Pl. XXI, Fig. 1). As the neck and perithecium develop, so the lid is pushed up through the limestone and the tissue above becomes disorganized, ultimately breaking away from the thallus (Plate XXI, Fig. 1 b). At the base of the developing perithecium there is a plate of inflated hyphae, which also becomes thick walled and dark coloured later on (Text-fig. 3 and Pl. XXI, Fig. 1). After the spores have been freed through the pore the wall shrinks and the old perithecium is washed or is blown out of the depression. This pit is not filled in with hyphae, as are cavities similarly left in aerial lichens, but the limestone lining the hollow is soon occupied by gonidial groups (Text-fig. 4).

I. A. a. *Lecidea immersa*, Ach.

The external appearance of *Lecidea immersa* is not unlike that of *V. calciseda*. There is the white or pale grey, granular surface, but except for the marginal regions fewer black dots, representing the external hyphal ends, are present. The black or dark-brown fruiting bodies appear larger than those of *V. calciseda*, and although the general shape of the apothecial area is circular, yet not uncommonly one finds them with an oval outline—probably this is the result of fusion of two apothecia developing closely together. After treatment with hydrochloric acid, the thallus of *Lecidea* is still very like *Verrucaria*, although it is thinner and only penetrates the limestone to a depth of 400μ . The vertical section also shows certain similarities, and because of this a detailed description will not be given. The main points of difference are as follows: The *cortex* is 34μ wide, and between the hyphal groups—even at the margin where the tissue is denser—there are fewer hyphae passing from group to group. Near the margin there are many hyphae with brown external tips.



TEXT-FIG. 5. *Lecidea immersa*. Young apothecium (longitudinal section).

The *gonidial* zone is wider, varying between 80μ and 100μ , but this is due to the fact that the clumps are somewhat scattered in the vertical direction. Apart from this the two gonidial zones are rather alike, except that, as in the cortex, there are fewer hyphae crossing from group to group.

The rhizoidal zone offers the most striking difference between the two species. In *L. immersa* there are no inflated hyphae in the thallus. Cells of about twice the width of the ordinary hyphae are present, but even these are very few. The hyphae of the rhizoidal region of *Lecidea* are very much narrower than those of *Verrucaria*; also, the width of the rhizoidal zone as a whole is considerably less in the former type.

The apothecium. Although from the surface the young state of the fruiting body appears very like the perithecium of *Verrucaria*, its construction is very different. This is clearly seen in the longitudinal section (Text-fig. 5). It is of the ordinary lecideine type and needs no description. The pit which has been formed during the development of the apothecium reaches down to about 200μ or more. In the tissue of the hypothecium there are many small crystals which have not been dissolved by the acid. Although inflated hyphae are not found in longitudinal section either in the young apothecium or in the thallus tissue, yet in freshly decalcified material mature

apothecia were found to possess numerous spherical oil cells, which usually occurred singly. These were readily seen when mature apothecia were crushed by slight pressure of the cover-slip. They occurred in the dense tissue of the hypothecium and in the region immediately below. As in *Verrucaria*, once the fruiting body has been removed, the walls of the pit are occupied afresh by gonidia and hyphae. Spermogonia are also found in small hollows, but these penetrate only to the base of the gonidial zone.

I. A. b. Lichen 'X' (not determinable).

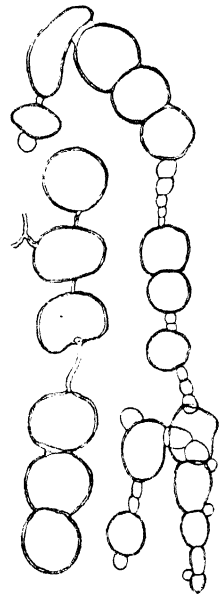
The surface of the thallus 'X' in the limestone shows nothing distinctive. There are no apothecia or perithecia and no black spots representing the external tips of hyphae. Very occasionally one finds the small blue-green algal colonies, but, as they are commonly found on endolithic lichen surfaces, they form no distinguishing feature. When decalcified the thallus surface appears very much smoother than that of either of the types described above, and there is also a greater thickness, the lichen penetrating the rock to a depth of 1,000 μ –1,100 μ .

In vertical section the three zones are clearly distinguished. For some time the *cortex* was thought to be of a very loose texture. It was only when the sections had been deeply stained with Congo red, in addition to the haematoxylin, that the very fine delicate walls of the majority of the hyphae of the cortical zone could be detected. The cortex measures 46 μ –70 μ , which is wide when compared with *Lecidea immersa* and *V. calcisceda*. It seems to be composed of two kinds of hyphal filaments: (i) the smaller number, made up of wide quadrate cells possessing dense contents which stain deeply with haematoxylin; (ii) the larger number, whose contents do not stain, the presence of these only being betrayed by the faint pinkish tinge of their walls (Pl. XXI, Fig. 3). The hyphae with the dense contents can be traced very easily from the outer edge of the thallus to the gonidial zone. In this region they appear to pursue a slightly tortuous path, branch but little, and generally preserve a direction at right angles to the surface of the rock. Usually the end one or two cells are colourless and correspond to the second type of hyphae. Filling in nearly all the spaces not occupied by the above hyphae are those filaments the cells of which appear to be without contents. At the outer limit of the thallus, together with the end cells of the former type, they seem to form a fairly continuous layer, which may be slightly brownish on the extreme outside.

The *gonidial zone* measures on the average 92 μ –140 μ . This great width is due to the fact that the narrow gonidial groups are rather elongated in the vertical direction. The size of the groups varies considerably (compare Text-figs. 7 and 8 with Pl. XXI, Fig. 3). The gonidia, both as clusters and individuals, are spun round by both kinds of hyphae, but more

particularly by those with dense contents. Those in the gonidial region are much narrower than similar hyphae in the cortex. From each of the little clusters filaments branch off and cross over or pass down to the neighbouring groups when the latter are situated some distance apart. When the clusters are close together these hyphae pass downwards, forming a dense strand of parallel filaments.

Rhizoidal zone. From the gonidial zone hyphae of both kinds pass downwards, branch very freely, and appear to form a dense network. In this region of transition from the upper zone to the rhizoidal region nearly all the hyphae appear to have the power of producing small inflated cells. These may be formed in continuous rows, or with short lengths of narrow hyphae in between. They resemble very closely the 'empty' cells of the cortical and gonidial zones. From this mass of hyphae spring the 'rhizoids'. These are narrow filaments, except when inflated to 14μ or thereabouts, with cross-walls far apart. They contain small globules of oil which is of the same nature as that found in the spherical dilations. Instead of the swellings occurring singly at the end of filaments as in *V. calciseda*, they are seen in 'X' to be most frequently in simple chains (Text-fig. 6) and sometimes in very small clusters. Frequently in a longitudinal section one can see that the structure of the rock has some influence on the rhizoids. The parallel lines of cleavage of large calcite crystals are often followed by branches of smaller inflated hyphae which join on to the main filament at an angle of 60° .



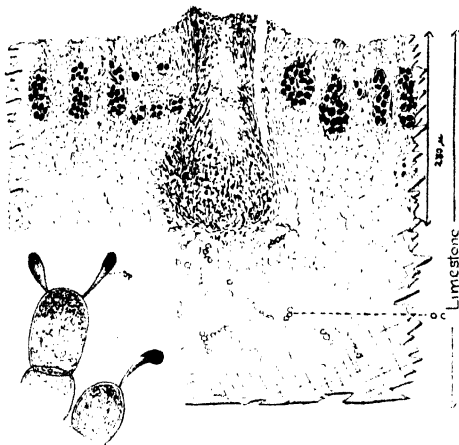
TEXT-FIG. 6. Lichen 'X'. Inflated hyphae.

Reproductive organs. Ascus-bearing bodies and asci have not been found in lichen 'X', but there are two kinds of spore-bearing organs apart from these. Both form pits which penetrate the limestone to more than twice the depth of the gonidial zone.

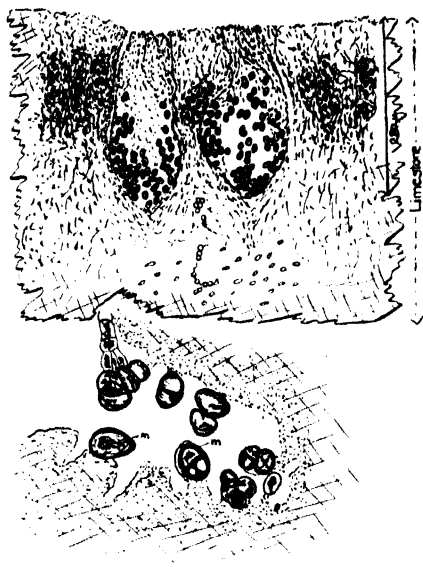
Spermogonia. The first kind one recognizes as simple immersed spermogonia (Text-fig. 7) bearing on their infolded walls countless spermatia of the club-shaped type. Numbers of these spermogonia have been cut both horizontally and longitudinally. At the apex the neck is narrow and generally circular in cross-section and measures about 70μ in diameter; if oval in outline the measurements are about $55\mu \times 180\mu$. Lower down in the rock where the spermogonium reaches its greatest breadth the average measurement is 200μ .

The second spore-bearing bodies appear very like pycnidia, but they bear *macrospores*. In general appearance they resemble the spermogonia, but

they are without the dense lining of very small hyphae. The spores, however, are very different from spermatia (Plate XXI, Figs. 4, 5, 6, and 7, and Text-fig. 8). They are borne on the hyphae lining the cavities and generally occur in such masses that it is difficult to see their actual origin. Since the spores are so large, 5μ or more, the size of the cavity itself is very much reduced. They (Plate XXI, Figs. 4, 5, 6, and 7) are at first colourless and apparently thin walled, but later become thick walled and dark brown. In the younger stages one is able to see large projections into the cell cavity (Plate XXI, Figs. 6 and 7). Apart from such projections as appear almost



TEXT-FIG. 7. Lichen 'X'. Spermatogonium; *a.c.* = inflated oil cells. Inset: *Sp.* = spermatium.



TEXT-FIG. 8. Lichen 'X'. Pycnidia with macrospore. Inset: portion of wall bearing macrospores. *m* = macrospore.

to divide a cell into two, the spores may be uniseptate (Plate XXI, Figs. 6 and 7), though often they are simple (Plate XXI, Fig. 4) and without projections into the cavities.

Tulasne (7) has recorded the presence of both spermatogonia and pycnidia on the lichen thallus. The pycnidiospores or stylospores were described as 'larger bodies than spermatia, occasionally septate, and containing oil drops or guttulae. These spores are pyriform or ovoid in shape and are always borne at the tips of simple sporophores' (8). In the case of lichen 'X', also, the two kinds of spores are quite distinct.

I. A. c. Placodium rupestre, var. *calvum*, forma *incrustans*, A. L. S.

Thalli of *Placodium rupestre*, var. *calvum*, forma *incrustans*, are small, being about one square centimetre in area. Nevertheless, they are often

crowded with small yellow immersed apothecia. To the naked eye the thallus appears white, but under the microscope the surface is seen to be speckled with black or dark-brown spots and patches. It is granular and more irregular than in the three types previously described. After scratching away the superficial layers of limestone, comparatively large, bright green nests of gonidia are exposed. The apothecium originates within the rock, as in *Lecidea immersa* and *V. calciseda*—the limestone above gradually breaking down, caving in, and falling on the top of the young apothecium. This accounts for many of the hollows appearing too big for the young apothecia.

After decalcification the upper surface of the thallus appears coarsely woolly, the comparatively large white clumps of tissue being rather far apart and apparently having little or no connexion with each other. From the underside as well as from the upper, the apothecia can be seen; in the former case they appear as hemispherical, pale yellowish, protruding lumps. The thallus is very thin, the average thickness being 160μ , and there are only a very few extremely short rhizoids. The small black spots on the surface have no special significance. Occasionally a brownish patch caps one of the white clumps of tissue, but this feature is not common.

In vertical section several striking differences from the former types are seen. The thallus, as indicated above, is made up of a number of almost isolated masses of tissue (Plate XXI, Figs. 8 and 9). At the top of each mass, which is situated in a pocket in the limestone, there is a more or less solid layer, about 23μ thick, of quadrate hyphal cells. This acts as a plug protecting the tissue below, which is composed of hyphae and gonidia—the latter being scattered about without any definite grouping of the cells (Plate XXI, Fig. 9). The algal cells are unusually large for the size of the thallus. From below arise a few hyphae which, for want of a better term, may be called rhizoids, and in these there are no inflated cells. In a typical case (Plate XXI, Fig. 9) from the outside of the 'cap' to the base of the pocket measures 100μ , and including the rhizoids 125μ may be reached. The whole mass is usually narrower towards the outside of the limestone, being only 46μ in diameter, while at the base it widens or branches and may have a breadth of 92μ or more. Very few hyphae link up these gonidial groups, so that nowhere does one find either a continuous cortical, gonidial, or rhizoidal zone.

The apothecia usually penetrate the limestone to a depth below the base of the gonidial pockets. They seem to have no more connexion with their thallus than have the parts of the thallus one with another.

From the above description it is seen that *P. rupestre*, var. *calvum*, forma *incrustans*, almost merits a position in the B (*homoiomorous*) section. Against this position there are, however, the caps of hyphal cells above the gonidia which correspond to the cortical tissue in the other endolithic lichen

thalli—particularly those of section *a* in *A*—and there is, in addition, the very poorly developed rhizoidal system.

I. B. a. Lichen 'Y' (an indeterminate form, since no fruiting bodies have been found).

A detailed description of this species will not be given, since Bachmann (loc. cit.) has already described a similar form, but as he did not include any detailed drawing of a decalcified section of the thallus as a whole, the present writer ventures to insert this form as illustrating a homoiomerous thallus, and also because in this type there are several points of interest apart from the actual structure of the thallus.

While still embedded in the rock, lichen 'Y' has a dark rusty grey colour with numerous black spots, but after decalcification it appears golden brown. The thallus is very thin, measuring only 140μ – 200μ . No rhizoids are present. In vertical section (Plate XXI, Fig. 10) there is a discontinuous band of dark-coloured hyphae = X, dead *Trentepohlia* cells = Y, and many dark bodies = Z, which appear very like fungal spores. These 'spores' lie on the surface of the thallus and send down into the rock hyphae which wind round the gonidial constituent of the lichen. In fact many cases of this kind have been seen and many of the hyphae of the thallus originate in this way. (Plate XXI, Fig. 13, shows a 'spore' at the surface giving rise to hyphae which encircle the *Trentepohlia* cell already in the rock; the dotted region represents the limestone.) Dark hyphal cells which lie on the surface put out branches into the rock; these branches are also thick walled and dark coloured, but, where in contact with the gonidial cells, their walls are always colourless and thin. Below the main masses of dark-coloured hyphae and algal cells one usually finds many filaments of *Trentepohlia*, encircled by hyphae, penetrating the rock. Lower in the limestone the gonidia with their attendant hyphae are less common, and at about 200μ below the surface they are absent. Sometimes many *Trentepohlia* filaments lie parallel with each other, particularly near the surface of the limestone, yet the structure of the rock seems to play no part in the structure of the thallus, for the gonidial filaments and the hyphae, when the latter occur alone, lie in the limestone at all angles with the surface.

Generally it is the *Trentepohlia* which penetrates farthest into the rock, either alone or partly surrounded by hyphae; even in the latter case that part of the algal cell farthest in the rock is usually free of hyphae (Pl. XXI, Figs. 11 and 12). Since hyphal filaments are found in the rock without gonidia, one must conclude that they too are capable of the pioneer boring of the limestone. This is also clearly indicated by the penetration of the rock by the germinating filaments of the 'spores' at the surface.

II. A. *Aspicilia calcarea*, Koerb. (*Lecanora calcarea*, Sommerf.).

Bachmann (loc. cit.) has described *Aspicilia calcarea*, but only the endolithic part of the thallus. By his method of preparation of sections of endolithic lichens it is impossible to obtain the whole depth of the thallus in one section, but by the method adopted in this investigation it is a very simple matter to section the endolithic and epilithic parts of the thallus in their relative positions. Because such a section has not previously been prepared, it was thought to be advisable to add a few remarks on this type. Fragments of limestone of about 1 cm. in thickness, and having on their surface the crustaceous or epilithic part of *A. calcarea*, were chiselled from the surface of the rock, immersed in dilute hydrochloric acid, and the whole process then carried on exactly as before. Since this species grows fairly rapidly over the substratum, care must be taken not to confuse disorganizing endolithic thalli, when they are present, with the endolithic part of *Aspicilia*. In a longitudinal section of a fully developed thallus the epilithic part is seen to vary in thickness from 190μ to 250μ , and to consist of cortical, gonidial, and medullary zones of a normal type (Pl. XXI, Fig. 14). The endolithic part is composed of gonidial groups and rhizoids, the latter apparently consisting of two kinds—thin and thick. The 'transition' zone (Pl. XXI, Figs. 14 and 15) of the upper to the lower part is represented by a dense mass of hyphal cells which are slightly enlarged and whose contents are very small and pressed closely against the walls (Pl. XXI, Fig. 15). In the part of the crustaceous thallus immediately above these cells and directly connected with them are roundish hyphae, some of which appear empty while others have contents of the usual type, i.e. not pressed against the cell-wall. From this zone of slightly enlarged, freely branching hyphae arise (i) the rhizoids, (ii) the hyphae which encircle some of the endolithic gonidial clusters, and (iii) the hyphae which form the large clusters of swollen spherical cells in the upper layers of the rock (Pl. XXI, Fig. 14, and Text-fig. 9). The fine colourless rhizoids are about 2μ wide and arise from the ends of such filaments as are represented in Pl. XXI, Fig. 15, *ph*. As Bachmann (loc. cit.) has stated, they are far more numerous than the wider yellowish ones, which are about 5μ in diameter. The former branch freely and twist about in the substratum, so that in a longitudinal section only short portions of them are seen. They contain oil globules and form small clusters of inflated cells of about 6μ diameter, which may be found alone or in connexion with the gonidial groups. Lower down in the limestone these narrow hyphae show a tendency to associate in strands, and remind one very strongly of the strands of 'oil hyphae' figured by Lang (loc. cit.) for *Biatorrella simplex*. In longitudinal sections simple chains of inflated hyphae have not been discovered, but

these have been found in the freshly decalcified material without sectioning. They measured at least $12\ \mu$ in diameter and arose from hyphae similar in every respect to the ordinary *Aspicilia calcarea* type. The simple chain of inflations resembled very closely those described for lichen 'X', and since they have not been seen in sections of *Aspicilia* one feels very doubtful whether they belong to that form.

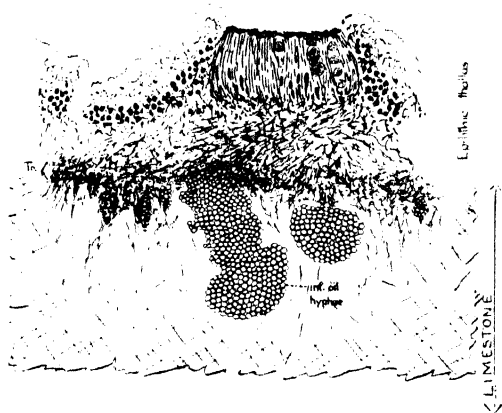
The wider rhizoids are unevenly distributed and their origin from the hyphae of the crustaceous part of the thallus seems a little obscure. This, together with the fact that they are usually brownish or yellow, makes one doubt their relation to *A. calcarea*, although they are present in most of the sections of *A. calcarea* in the limestone immediately below the epilithic thallus. Bachmann's description of the yellow hyphae found by him below the thallus of this species agrees with those of the present investigation.

The rhizoids penetrate the limestone to a depth of $2,800\ \mu$, making the width of the whole thallus over $3,000\ \mu$.

Occasionally algal cells are found in the 'transition' zone, but apart from these and the gonidial region of the epilithic thallus, there is the extraordinary development of gonidial groups in the limestone, where they may penetrate to a depth of $280\ \mu$ (Pl. XXI, Fig. 14). If they occur near the surface of the limestone, they are spun round by hyphae which are directly connected with the 'transition' zone: if lower down in the rock, they are encircled by thin-walled rhizoids. In some cases cells with dense contents surround the gonidia; in other cases slightly inflated hyphae with little or no deeply staining contents (Pl. XXI, Fig. 16), and sometimes a mixture of the three, may take part in encircling the algal cells (Pl. XXI, Fig. 17). Generally these groups occur as isolated rounded masses in the rock, and in this respect remind one of the *Placodium* type of endolithic thallus described. Occasionally these groups are connected vertically with each other (Pl. XXI, Fig. 14), but very rarely is there any lateral communication between them.

Yet another peculiarity obtains in the upper limestone layers below the crustaceous part of *A. calcarea* thallus. Certain thin rhizoidal hyphae, arising in the 'transition' zone in the usual way, penetrate the limestone and immediately branch very freely and form numerous inflated cells in the same way as that indicated for the hyphae of the 'transition' zone in Pl. XXI, Fig. 15. This branching of the hyphae and swelling of the cells goes on until huge clusters of spherical hyphae are formed. Bachmann (loc. cit.) has described the formation of these masses as follows: 'On one of the hyphae there arise, close together, numerous short branches. These go on growing parallel to each other and at right angles to the main hypha, projecting from it in all directions. A dilation of each of the cells of the entire branching system, not excluding those of the main hypha, goes on at the same time, until the spherical shape is attained. As a result of these

processes the spherules are brought into immediate contact with each other, the whole mass constituting a complex of cells having the appearance of a bunch of grapes in which it is no longer possible to distinguish the cells of the principal hypha from those of the lateral branches.' As Bachmann (loc. cit.) states, they are most frequently found in the uppermost layers of limestone (Pl. XXI, Fig. 14), but since he had not the epilithic part of the thallus *in situ* he was not able to see the relative position of the largest and more frequent clusters. In the present investigation by far the largest and most conspicuous masses of inflated hyphae are found in the limestone beneath or in the immediate neighbourhood of the apothecia



TEXT-FIG. 9. *Aspicilia calcarea*, showing apothecium and large clusters of inflated cells.
Tr. = 'transition' zone; inf. oil hyphae = complex mass of inflated oil hyphae.

which occurs in the epilithic part of the thallus (Text-fig. 9). In such clusters the spherical hyphae have a diameter of not more than 6 or 7 μ . It should be mentioned, both as regards the masses of spherically inflated cells and the endolithic gonidial groups, that they are not equally abundant in all parts of the thallus; in fact, in many parts they may be completely absent.

GENERAL DISCUSSION.

One of the most striking facts about the endolithic lichens here described is that, although they are embedded in the rock, their thalli have a structure similar to that of the sub-aerial forms to which they are related. For example, the genus *Lecidea* includes both sub-aerial and endolithic species, and both exhibit the heteromerous structure. In both types growth in area goes on at the circumference, where the thalli are thin and consist of fungoid elements alone. Towards the centre, where they are fully developed, the thalli present the typical heteromerous structure. The

limestone does not seem to hinder in any way the general arrangement and growth of the endolithic lichens. In epilithic thalli it is difficult to find a parallel to that presented by *Placodium rupestre*, var. *calvum*, forma *incrustans*, unless one could compare with it the small isolated squamules of the primary thallus of a *Cladonia*. The structure of certain species of epilithic and endolithic lichens, which have the filamentous *Trentepohlia* as their algal constituent, may also be compared. For example, *Coenogonium ebeneum* has a black-coloured, filamentous epilithic thallus, the hyphae encircling the erect *Trentepohlia* filaments. In the endolithic form termed 'Y', the algal filaments penetrate the rock, where they are subsequently surrounded by fungal hyphae in a manner exactly similar to that which obtains in *Coenogonium*. Consequently 'Y' could be regarded almost as an endolithic filamentous form, the filaments of which, instead of growing upwards into the air, penetrate downwards into the limestone, growing in length by an apical cell (Pl. XXI, Figs. 11 and 12) similar to that of the aerial form.

The collective mass of gonidia and hyphae in an ordinary lichen thallus is not usually formed from a single spore and a single algal cell, but from several masses of tissue, each starting from a separate growth centre. A similar development of the thallus obtains in endolithic forms. Spores falling on the surface of the rock may send out germinating tubes into the limestone. If the spore belongs to the same species as the endolithic thallus already present, then the hyphae germinating from the former will contribute to the formation of that thallus. This has been observed in several cases in lichen 'Y' (Pl. XXI, Figs. 10 and 13). In one or two old perithecial pits of *Lecidea immersa*, where re-colonization of the limestone lining was going on, dark-coloured rounded spores have been observed germinating and sending their hyphae into the cortical tissues of *Lecidea*. Though these hyphae resembled very closely those forming the cortex, yet the spores were not those of *Lecidea immersa*, which are oval in shape and colourless. The fate of such foreign hyphae in a thallus is not known, but the idea suggests itself that they may be the starting-point of a more vigorous species which will oust the slower-growing *Lecidea*. Foreign spores are often found on the surface of the endolithic thalli, where, as a rule, they do not germinate, but merely contribute to the speckled appearance of the surface.

Observations on the infection of the marginal regions by gonidia belonging to the Chlorophyceae have not been made. Since the gonidia are present in little clusters in pockets in the rock, the usual method of spreading the gonidia in the lateral direction, in which the hyphae first separate and then push the algal cells apart, would scarcely seem to hold.

The endolithic lichens undoubtedly obtain protection both from excessive drought and extremes of temperature; yet, from the fact that

the thalli exhibit the structure of ordinary epilithic forms while embedded in the rock, it may be assumed that the ordinary life of the organisms is carried on unhindered by the enclosing limestone. From the nature of the habitat it would seem that neither the cortex nor the rhizoidal zone is essential for the performance of the simple and obvious functions of protecting the gonidial layer and of fixing the plant to the substratum. In view of this, one may be permitted to conclude that both in epilithic and endolithic forms, unless it is a matter of inherent tendency on the part of the lichen to produce them, both cortical and rhizoidal zones have definite functions to perform, apart from the simpler ones mentioned above.

A feature which is present in most, but not all, endolithic lichens is the development, more particularly in the rhizoidal region, of spherical oil cells. Oil globules, however, are found in the rhizoids of all the endolithic species examined, whether they possess the inflated cells or not. The oil cells occur singly or in pairs, as in the terminal inflations of *V. calciseda*, in simple chains, as in lichen 'X', or in large complex cell-masses such as are found in the immersed part of *A. calcarca*. Similar oil hyphae have been found by Rosendahl (9) and Bachmann (10 and 14) in a few lichens which do not grow on a calcareous substratum, but these may be regarded as exceptional cases. It may be helpful at this point to summarize the cases where these inflated cells occur in the thalli described, and also their relative abundance.

Verrucaria calciseda. The rhizoidal and marginal regions, where they are very common. The young perithecium (lid and basal part).

Lecidea immersa. Apothecial tissue—common.

Lichen 'X'. Rhizoidal region—common.

Aspicilia calcarca. Rhizoidal region, more particularly in the neighbourhood of the apothecia.

Placodium rupestre, var. *calvum*, f. *incrustans*. Absent.

Lichen 'Y'. Absent.

Since they are not present in all forms and in all parts of the thallus they cannot be considered essential for the endolithic habit nor a characteristic of endolithic thalli, though the oil itself, which is common to the hyphae—more particularly the rhizoidal hyphae—may be an essential constituent of the thallus tissue. If one considers the position of the inflated oil cells in the types described, one finds that they occur in regions of special growth. The thallus of *V. calciseda* is one of the more rapidly growing species. It has been observed during the present investigation to oust from their positions *P. rupestre*, var. *calvum*, forma *incrustans*, and *L. immersa*, both of which are found to be without inflated cells in their thallus tissues. Fünfstück (loc. cit.) found inflated oil cells of *L. immersa* 8 mm. below the limestone surface, but in the thalli examined in the present

investigation *L. immersa* only penetrates to a depth of 1,100 μ , and no inflations, of the complicated type figured by Fünfstück, are found. Fünfstück states that *Opegrapha saxicola* is one of the richest in oil cells. He also says in another connexion that *O. saxicola* and *V. calciseda* are rapidly growing forms. The rate of growth of lichen 'X' is not known. It is interesting to note, in connexion with the development of the perithecia of *V. calciseda* and the apothecia of *L. immersa*, that oil cells are prominent. It is also curious that, even in the case of *A. calcarea*, where the apothecia are in the epilithic part of the thallus, there is in the rock below an unusual formation of these cells full of oil. It may be worth mentioning as a characteristic feature of the genus that during its early development the apothecium remains immersed in the thallus tissue. In regions of growth, or any other plant activity, carbon dioxide is given off, and in regions of rapid or locally concentrated growth one would expect a proportionally increased evolution of the gas. In these endolithic lichens it is in such regions that one finds the main masses of oil cellules, and it is in these same places that the more rapid solution of the limestone goes on. It seems natural to conclude, therefore, that growth, formation of oil, evolution of carbon dioxide, and solution of the limestone are intimately related. If this is so, then one can suggest a solution of the phenomenon of limestone penetration by these lichens. Such a solution would also explain penetration of the rock by *Trentepohlia* and moss rhizoids.

Various theories have been advanced to explain the use of the fatty material. It was stated by Zukal (15) to be a food reserve in case of drought or extra strain on the resources of the lichen. This has been disproved by Fünfstück's (loc. cit.) experiments on *V. calciseda* and *O. saxicola*. The researches of Beyerinck (12), Wehmer (13), and Stahel (14) all go to show that the formation of the fatty material is due to adverse conditions, such as absence of nitrogen or excess of calcium carbonate, while respiration is actively proceeding. Some of the material used in the present investigation had been kept dry and in the dark for two or four years, and it is interesting to note that the inflated cells and hyphae were full of oil at the end of those periods. As stated by Miss Lorrain Smith (8), the results obtained by the above investigators show how the fatty substance may be formed in those lichens which are deprived of nitrogen and have an excess of carbon dioxide which is not readily removed. This is clearly brought out in the present investigation, for wherever carbon dioxide evolution is at its greatest, there one finds the greatest formation of inflated oil hyphae. Furthermore, in the slower-growing species, inflated hyphae are absent, the oil being stored as small globules in the ordinary narrow hyphae. From the above considerations one can conclude with Fünfstück (loc. cit.) that the oil is a waste product formed under the special endolithic conditions.

Various theories have also been put forward by different investigators

to explain the boring action of the fine hyphae into the limestone, but they have all been discredited. That put forward by Wallroth (16) may be instanced. He stated that the rhizoids excreted an acid fluid which dissolved the limestone, the remainder of the thallus being raised in the air. Owing to the precipitation of calcium carbonate in the intercellular spaces of the thallus, the latter was so highly charged with the carbonate that it became as hard as stone. A consideration of the results of this investigation has led the writer to form the following hypothesis to explain the boring action of these lichens. The boring action is performed by the carbon dioxide of respiration, dissolved in the water which soaks down from the surface, and is retained by capillarity in the numerous cracks and pores in the limestone. This carbonic acid acts on the rock forming the soluble bicarbonate. This solution diffuses up, between the hyphae and the walls of the cavities made by them, to the surface film of water. From the surface it may either be washed away by the next shower of rain, or, in the case of drought setting in, it may precipitate the more stable carbonate on the surface. The continual precipitation of calcium carbonate on the surface of the embedded lichen will account for the permanent granular appearance of the surface of the endolithic thallus examined. There are numerous small depressions on the flat tables of limestone, and in these rain-water tends to collect. If this water is allowed to remain for a short time, it is found to contain calcium carbonate in solution. In the cases under discussion, since there is no chance of water, already containing calcium carbonate, draining into the depression, the carbonate must be derived either from the solution of the granules of limestone on the surface of the endolithic thalli, or by diffusion into the water of the bicarbonate produced by the boring action of the endolithic lichens lining the depression, or from both these sources. The rain-water probably contains a small amount of atmospheric carbon dioxide in solution, and this, together with the carbon dioxide of respiration of the rock lichens, is sufficient to dissolve the granules of limestone deposited on the surface as described above. If the small collection of water dries up, the dissolved limestone will be reprecipitated on the thalli, but in the case of a shower of rain it will be washed down into the neighbouring crevices; since the rainfall in these regions is very high, this will frequently take place. This will also explain the removal from the apothecial pits of the undissolved particles of limestone, for the processes which go on on a large scale in the depressions are also in progress in the minute apothecial hollows.

One has already come to the conclusion that the carbon dioxide of respiration of lichens on other substrata than limestone, e.g. shale, is responsible for a considerable proportion of the decomposition of the rock in their immediate vicinity. Miss Mellor (17) also has recently published a paper proving that the carbon dioxide of lichen respiration in solution

in the condensed water vapour of the atmosphere plays a considerable part in the decomposition of glass.

SUMMARY.

1. The investigation suggested itself during a general study of cryptogamic ecology, and this paper is confined to the examination of certain endolithic limestone lichens.

2. The method of preparation of material for study was to decalcify, with dilute hydrochloric acid, the lichen thallus as a whole, then wash it free of acid, fix, dehydrate, embed, cut serial sections, stain, and mount in the usual way.

3. The classification adopted is based on the thallus structure.

4. Endolithic lichens have a structure similar to that of sub-aerial forms.

5. The fruiting bodies originate within the rock—the deep pits being formed during development.

6. In regions of special growth numerous spherically inflated hyphae containing oil are found. In these same regions the solution of the limestone is more rapid. There is probably some relation between growth, evolution of carbon dioxide, oil formation, and solution of the limestone.

7. Inflated oil hyphae are absent in slower-growing species, but oil globules in the ordinary hyphae are common to all forms.

8. The oil is not stored food, but waste material produced under adverse conditions during the evolution of carbon dioxide; consequently there is an increased output of oil where growth is specially active.

9. It is suggested that the boring action is brought about by the carbon dioxide of respiration dissolved in water. The dilute acid attacks the rock, the soluble bicarbonate being formed. This is precipitated at the surface as the stable carbonate. The precipitation causes the granular appearance of the surface of endolithic thalli. The granules are constantly replaced by the fresh precipitation of calcium carbonate.

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EXPLANATION OF PLATE XXI.

Illustrating Mr. Fry's paper on Some Types of Endolithic Limestone Lichens.

Fig. 1. *Verrucaria calciseda*. Vertical section of thallus and perithecium (depth in limestone, 670 μ).

Fig. 2. *V. calciseda*. Vertical section of black marginal region between *V. calciseda* and *Placodium rupestre*, var. *calvum*, forma *incrustans*.

Fig. 3. Lichen 'X'. Vertical section of thallus (depth in limestone, 1,000-1,100 μ).

Fig. 4. Lichen 'X'. Macrospore.

Fig. 5. Lichen 'X'. Macrospore.

Fig. 6. Lichen 'X'. Macrospore.

Fig. 7. Lichen 'X'. Macrospore.

Fig. 8. *Placodium rupestre*, var. *calvum*, forma *incrustans*. Low power. Vertical section of thallus and apothecium (depth in limestone, 160 μ).

Fig. 9. *P. rupestre*, var. *calvum*, form *incrustans*. Vertical section of part of thallus.

Fig. 10. Lichen 'Y'. (Gonidia, *Trentepohlia*). Vertical section of thallus (depth in limestone, 140–200 μ).

Fig. 11. Lichen 'Y'. Gonidial cell from lower part of thallus.

Fig. 12. Lichen 'Y'. Gonidial cell from lower part of thallus.

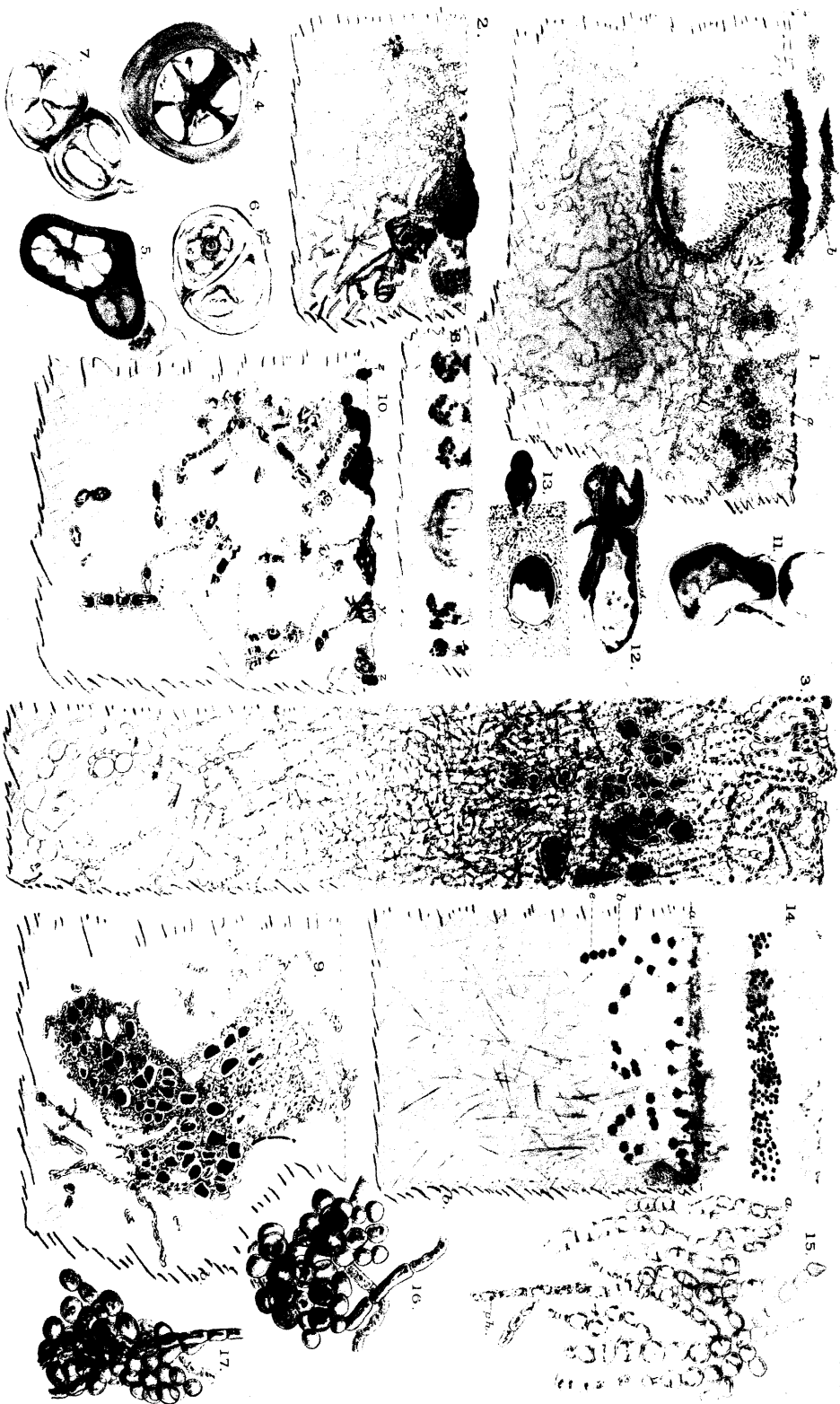
Fig. 13. Lichen 'Y'. Lichen spore germinating on surface of limestone and sending hyphae into the rock, which attack the gonidium. (Dotted area indicates limestone.)

Fig. 14. *Aspicilia calcarea*. Vertical section of thallus (width of whole thallus, 3,000 μ). Depth in limestone, 2,800 μ . Width of epilithic thallus, 190–250 μ . *a.* 'Transition' zone at top of limestone; *b.* Endolithic gonidial group; *c.* Small group of inflated cells; *d.* Four gonidial groups in vertical connexion.

Fig. 15. *Aspicilia calcarea*. A few enlarged hyphal cells from the 'transition' zone.

Fig. 16. *Aspicilia calcarea*. Endolithic gonidial group enlarged. Gonidia surrounded by small inflated cells.

Fig. 17. *Aspicilia calcarea*. Endolithic gonidial group enlarged.



On the Influence of Immersion in certain Electrolytes upon Cells of *Saxifraga umbrosa*.

BY

MAUD WILLIAMS, B.Sc.

With three Figures in the Text.

IN earlier work (1) upon this subject strips from the upper side of the leaf-stalk of *Saxifraga umbrosa* were immersed in electrolytes of known concentrations and the times of immersion required to produce a certain arbitrary change determined. The cells in question are rich in tannin, and over a long period are impermeable to weak ferric chloride solution, so that undamaged cells do not show the blue coloration due to reaction of tannin and iron chloride when the sections are mounted in the latter solution. When, however, immersion in the electrolytes studied had been prolonged sufficiently the nature of the protoplasm was found to be so modified that the ferric chloride diffused it, and it was possible to obtain blue tinting and later a precipitate. In the experiments already recorded immersion in the electrolyte was continued until it was found possible to produce the ferric chloride reaction (using 0.2 per cent.) when the sections were soaked in the reagent for three minutes.

The experiments showed that all the solutions studied produced this change if the time of immersion were sufficiently prolonged, and there was evidence for a definite connexion between time required and the concentration employed. Over the limited range of time and concentration it appeared that the following formula held for a particular electrolyte:

$$\log T + k (\log C + 1) = \text{constant}$$

where T = time required,

C = concentration in gram-mols. per litre,

and the constant depended upon the electrolyte.

These experiments were open to criticism from several points of view.

1. The experiments had been carried out at room temperature and no corrections were possible for fluctuations.

2. It had not been determined whether the change in the protoplasm was of a reversible nature or whether permanent injury had resulted.

3. The effect of the ferric chloride upon the protoplasm had not been studied beyond the fact that the solution alone took several hours to enter

healthy cells. Since the iron salt used was one liable to hydrolyse, further study would have been advisable.

After the results had been published it was found that the formula agreed with one already derived by other workers for definite measures of change in the case of bacteria (2) and unicellular Algae (3). The additional interest of this similarity led to the decision to repeat and extend the work, using a more stable reagent for testing the change in the protoplasm and carrying out all immersions at a fixed temperature.

CHOICE OF REAGENT.

Search was made for a reagent which would prove a delicate test for tannin and at the same time would be stable in dilute solution. Dekker (4), in his work on tannins, recommends a 5 per cent. solution of potassium dichromate for staining purposes.

It was found that in the particular cells studied a solution of 0.1 per cent. was sufficient to produce a definite precipitate in a period of three minutes when the cells had previously been rendered permeable. This very dilute solution was also suitable for sections studied under the ultra-microscope, as the first-formed particles came down with well-marked Brownian movement. The next step was to find whether this weak solution was stable. This was done by a series of measurements of the resistance when a conductivity cell was filled with the solution and the whole kept in a thermostat at 25°C. The measurements were made by the 'metre bridge' method, a commutator and galvanometer being employed. The readings were taken at different times extending over forty-two days; for each date the mean of three determinations was taken. The values given in Table I showed a variation of only 1 per cent., so that the solution was considered free from objection.

All the tests on the plant material necessary in the work given below were made with solutions prepared from the same sample of crystals as those used in the conductivity tests.

The influence of various concentrations of the potassium dichromate upon the cell had next to be studied. The plant material, treated as for the rest of the immersions, was placed in tubes containing 30 c.c. of the solution and kept in a thermostat at 25°C. In each concentration the time was found when a clearly defined precipitate first appeared in the cells. The results are shown in Table II, and indicate that this salt has a 'time and concentration' connexion of the same type as that already mentioned, but that the influence of a 0.1 per cent. solution, acting upon the cell for three minutes, is negligible.

The effect of changing the temperature of immersion was also studied for the same salt, a 3 per cent. solution being chosen. The readings for time required and temperature are given in Table III and Fig. 1. Only

one experiment was carried out at 9° C. owing to the difficulty of maintaining a steady temperature by the means available, viz. the addition of small quantities of ice-cold water to the thermostat of tap-water. The tempera-

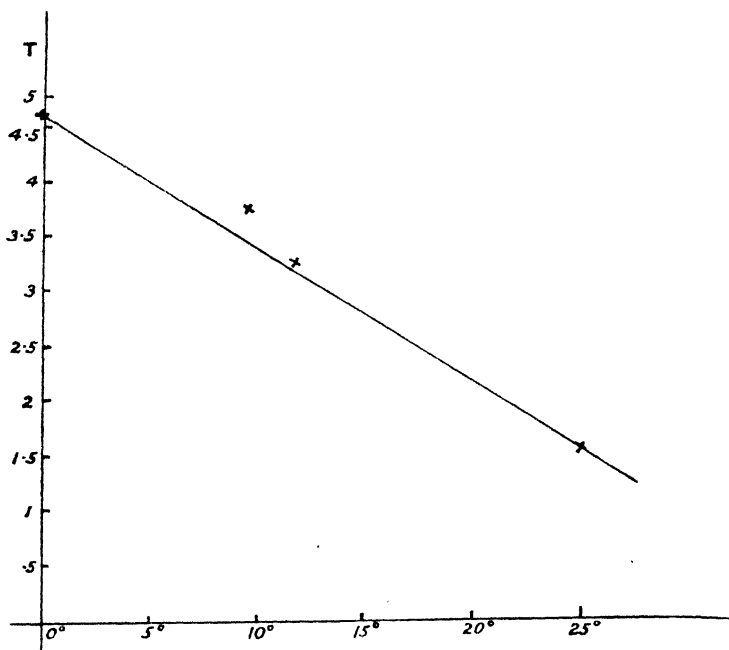


FIG. 1. Influence of change of temperature upon permeability to $K_2Cr_2O_7$. Concentration = 3 %. T = time in hours to produce precipitate.

ture effect showed the advisability of keeping strictly to one value throughout the tests, although the errors due to slight fluctuations of the room temperature in earlier work were probably small compared with those due to 'past history' factors.

PRELIMINARY TREATMENT OF MATERIAL AND IMMERSIONS.

The plants used for this portion of the work were all grown in the same soil. For winter use specimens were kept in a cool greenhouse and rosettes cut and kept in water in the laboratory for one day before the sections were made. Long strips were torn from the petiole and kept for twenty-four hours in a large quantity of distilled water, which was changed at intervals. It was found to be extremely important to select leaves of the same condition, and those from the base and apex had to be rejected. Doubtless the differences in the cell-walls modified diffusion. On account of lack of similar material in two or three of the tests quoted it will be found that fewer values than the usual number had to suffice.

On preliminary examination of the material with 1 per cent. caffeine it was found that a greater number of cells had tannin contents than had

been the case in the plants from another source used in the earlier work; consequently in the $K_2Cr_2O_7$ tests a general appearance of precipitate was sought.

It is important to record that no stirring was done during the immersions, and care was taken to remove sections at the necessary intervals with the least possible disturbance.

The temperature of the thermostat was read at intervals and the usual fluctuation during an immersion was 1 per cent.; in a few cases it amounted to 2 per cent.

OBSERVATIONS UPON THE NATURE OF THE CHANGE.

With a view to finding whether the change was of a reversible type, sections were closely examined after different stages of immersion and the following facts observed:

(a) With concentrations of salts sufficient to cause ordinary plasmolysis the nature of the surface underwent changes as immersion was continued. At first the surfaces were parts of smooth curves with the exception of the threads connecting small spheres; later, irregularities appeared and the protoplasm became more granular.

(b) With the weaker solutions a shrinkage was very slowly induced, and here again the protoplasmic surfaces showed irregularities.

(c) Circulation slowed down, and, in a thin section, had stopped in almost every cell at the stage when the reaction with the $K_2Cr_2O_7$ could be obtained.

(d) Sections immersed for the critical times did not recover their circulatory power or normal appearance upon even prolonged treatment with distilled water.

Further examination was carried out with the 'dark field' illumination produced by a paraboloid condenser. Material immersed for the critical times in KI or in KCl was used in these tests. Before immersion the sections showed, against the black field, light scattered from their cell-walls and from chloroplastids, very faint light scattered from the general protoplasm, and some bright particles, difficult to focus, near the surface of the protoplasm. These particles possessed Brownian movement.

With the same focus it was seen after immersion that movement had stopped, but there was much more general diffusion of light, denoting a state of greater aggregation. No further change was observed when the sections were left in distilled water.

When the $K_2Cr_2O_7$ was applied, particles in Brownian movement appeared in the corners of the cells as well as in connexion with the plasmolysed masses.

These facts, taken collectively, show that immersion for the critical time produces an irreversible change and the material may be said to be

'poisoned'; moreover, at the time the potassium dichromate is able to penetrate the protoplasm the tannin is able to diffuse out of the vacuole.

RESULTS.

The times of immersion (critical times) for the various concentrations are quoted in Table IV. As the curves for time and concentrations all follow the type already recorded they are not drawn, but the graphs for $\log T$ and $\log C+1$ are shown in Figs. 2 and 3. It will be seen that study has been made of a series of potassium salts and of three sodium salts, to try to discover whether anion or kation determines the relationship.

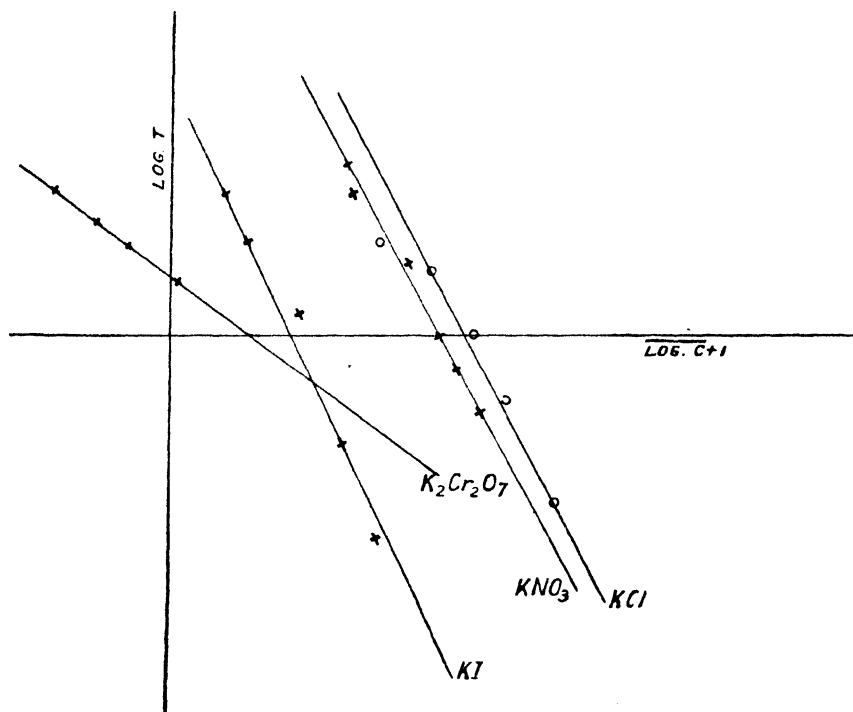


FIG. 2. K salts.

TABLE I.

Resistance of 0.1 per cent. solution $K_2Cr_2O_7$.
Electrodes = platinized plates.

Date.	Age of Solution	Temperature of Thermostat.	Mean value of Resistance.
1918.	Days.	Degrees Centigrade.	Ohms.
July 31	0	25	428.7
Sept. 2	33	25	429
Sept. 4	35	24.95	424.9
Sept. 9	40	25	428.7
Sept. 11	42	24.95-25	428.3

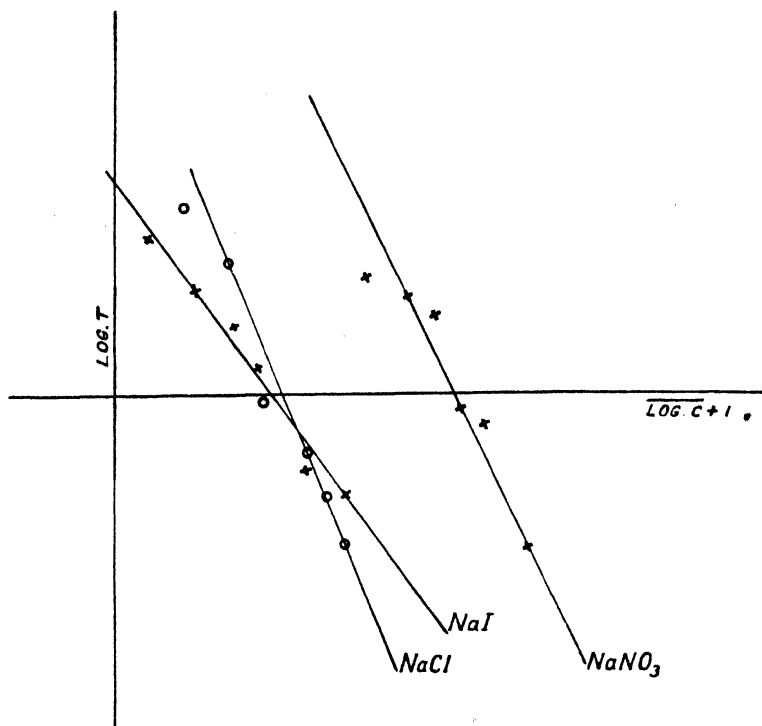


FIG. 3. Na salts.

TABLE II.

$K_2Cr_2O_7$. Temperature $25^\circ C$. Temperature error 1 per cent.
Concentration and Time of Immersion needed to produce precipitate.

Concentration in gram-mols. per litre.	Date.	Time needed in hours.	Average time.	Log T.	Log C + 1	Value of Log T + 0.67 (Log C + 1).
0.108	1919. Feb. 28	1.75	1.6	0.204	0.033	0.23
	Feb. 28	1.5				
	March 7	1.66				
0.072	April 4	2.16	2.08	0.318	1.837	0.21
	April 4	2				
0.054	March 12	2.5	2.66	0.425	1.73	0.24
	March 13	2.75				
	March 27	2.75				
0.036	March 28	3.25	3.33	0.522	1.536	0.23
	April 3	3.25				
	April 3	3.5				

Average value of constant in last column = 0.23. Maximum deviations from average = +4 %.
-8 %.

TABLE III.

Time of immersion required and temperature. Salt: potassium dichromate 3 per cent.

<i>Temperature.</i> Degrees Centigrade.	<i>Date.</i> 1919.	<i>Time.</i> Hours.	<i>Average Time.</i>
25	Feb. 28	1.75	1.6
	Feb. 28	1.5	
	March 7	1.66	
18.3	March 7	3	3.25
	March 18	3.5	
9	April 2	3.75	3.75
0	March 21	4.75	4.58
	March 27	4.25	
	March 28	4.75	

TABLE IV.

Critical times for various concentrations.

Temperature 25° C.

Concentrations in gram-mols. per litre.

Times in hours.

A. KNO_3 .

<i>Concentration.</i>	<i>Date.</i>	<i>Critical Time.</i>	<i>Average Time.</i>	<i>Log T.</i>	<i>Log C + 1.</i>	<i>Value of Log T + 1.77 (Log C + 1).</i>
1.42	1920. Jan. 14	0.42	0.52	1.72	1.15	1.75
	Jan. 27	0.5				
	Jan. 27	0.66				
	Feb. 2	0.5				
1.19	Jan. 20	0.75	0.77	1.89	1.07	1.78
	Jan. 21	0.83				
	Jan. 26	0.75				
0.98	Jan. 13	0.83	0.98	1.99	0.99	1.74
	Jan. 13	1				
	Jan. 14	1				
	Jan. 14	1.08				
0.74	Jan. 19	2	1.83	0.26	0.87	1.79
	Jan. 21	1.83				
	Jan. 26	1.66				
0.59	Jan. 26	2	2.08	0.32	0.77	1.68
	Jan. 27	2				
	Feb. 2	2.16				
	Feb. 2	2.16				
0.44	Feb. 11	4.25	4.25	0.63	0.64	1.76
	Feb. 11	4.25				
	Feb. 18	4.25				

Average value of constant in last column = 1.75. Maximum deviations from average = + 2 %.
- 4 %.

TABLE IV (*continued*).*B.* KCl.

<i>Concentration.</i>	<i>Date.</i>	<i>Critical Time.</i>	<i>Average Time.</i>	<i>Log T.</i>	<i>Log C + 1.</i>	<i>Value of Log T + 1.72 (Log C + 1).</i>
2.7	Jan. 6, '20	0.25	0.25	1.398	1.43	1.86
	Jan. 7, '20	0.25				
1.87	Dec. 9, '19	0.5	0.55	1.74	1.27	1.92
	Dec. 9, '19	0.58				
	Dec. 15, '19	0.58				
1.44	Dec. 15, '19	1	0.98	1.991	1.16	1.99
	Dec. 16, '19	1.08				
	Dec. 16, '19	0.83				
	Dec. 16, '19	1				
0.96	Dec. 8, '19	1.5	1.58	0.199	0.98	1.89
	Dec. 9, '19	1.5				
	Dec. 10, '19	1.75				
0.485	Dec. 15, '19	3.25	3.22	0.508	0.69	1.70
	Jan. 5, '20	3.33				
	Jan. 6, '20	3.08				

Average value of constant in last column = 1.87. Maximum deviations from average = + 7 %.
- 9 %.

C. KI.

<i>Concentration.</i>	<i>Date.</i>	<i>Critical Time.</i>	<i>Average Time.</i>	<i>Log T.</i>	<i>Log C + 1.</i>	<i>Value of Log T + 2.09 (Log C + 1).</i>
0.59	1919.		0.18	1.255	0.77	0.86
	Oct. 27	0.16				
	Oct. 28	0.25				
	Oct. 29	0.16				
	Oct. 29	0.16				
0.445	Oct. 22	0.5	0.41	1.613	0.65	0.97
	Oct. 27	0.33				
	Oct. 28	0.5				
	Oct. 28	0.33				
0.3	Oct. 15	1.25	1.24	0.093	0.477	1.09
	Oct. 28	1.33				
	Oct. 28	1.16				
	Nov. 23	1.25				
0.18	Oct. 13	2	2.25	0.352	0.255	0.89
	Oct. 7	2.25				
	Oct. 8	2.25				
	Oct. 15	2.5				
0.15	Nov. 3	3.25	3.33	0.52	0.176	0.89
	Nov. 3	3.5				
	Nov. 23	3.25				

Average value of constant in last column = 0.94. Maximum deviations from average = - 8 %.
+ 16 %.

D. K₂Cr₂O₇. See Table II.

TABLE IV (continued).

E. NaCl.						
Concentration.	Date.	Critical Time.	Average Time.	Log T.	Log C + 1.	Value of $\frac{\text{Log } T + 2.3}{(\text{Log } C + 1)}$.
	1919.					
0.68	June 13	0.33	0.27	1.43	0.83	1.34
	June 19	0.25				
	June 20	0.25				
0.59	July 17	0.4	0.37	1.57	0.77	1.34
	July 28	0.4				
	July 29	0.33				
0.51	June 26	0.66	0.61	1.78	0.71	1.41
	June 27	0.66				
	July 3	0.5				
0.34	June 13	0.83	0.89	1.95	0.53	1.17
	June 13	0.75				
	June 19	1				
	June 20	1				
0.255	July 3	3	2.9	0.46	0.41	1.40
	July 4	3				
	July 4	3				
	July 28	2.5				
0.17	July 28	4.5	4.75	0.68	0.23	1.18
	July 29	5				
	July 29	4.75				

Average value of constant in last column = 1.31. Maximum deviations = -10.6 %.
+ 7.6 %.

F. NaI.						
Concentration.	Date.	Critical Time.	Average Time.	Log T.	Log C + 1.	Value of $\frac{\text{Log } T + 1.38}{(\text{Log } C + 1)}$.
	1920.					
0.67	Sept. 21	0.42	0.42	1.62	0.88	0.77
	Sept. 21	0.42				
	Sept. 24	0.42				
	Sept. 24	0.42				
0.49	Sept. 6	0.5	0.5	1.7	0.69	0.65
	Sept. 7	0.42				
	Sept. 9	0.58				
0.33	Sept. 7	1	1.17	0.07	0.52	0.79
	Sept. 9	1.25				
	Sept. 22	1.25				
0.26	Sept. 21	1.75	1.7	0.23	0.42	0.81
	Sept. 21	1.66				
	Sept. 22	1.75				
	Sept. 22	1.66				
0.2	Sept. 10	2.25	2.31	0.36	0.30	0.77
	Sept. 10	2.42				
	Sept. 20	2.25				
0.13	Sept. 20	3.83	3.68	0.57	0.11	0.72
	Sept. 20	3.66				
	Sept. 27	3.5				
	Sept. 27	3.75				

Average value of constant in last column = 0.75. Maximum deviations = + 8 %.
- 13 %.

TABLE IV (*continued*).

G. NaNO_3 .						
Concentration.	Date.	Critical Time.	Average Time.	Log T .	Log $C+1$	Value of $\frac{\log T + 1.91}{(\log C + 1)}$.
1920.						
3.18	June 8	0.25	0.27	1.43	1.55	2.39
	June 14	0.25				
	June 14	0.33				
	June 14	0.33				
2.18	June 2	0.75	0.77	1.87	1.34	2.49
	June 2	0.75				
	June 6	0.83				
	June 6	0.75				
1.88	June 15	0.83	0.83	1.92	1.27	2.34
	June 16	0.83				
	June 22	0.83				
1.37	May 26	1.83	1.83	0.26	1.14	2.46
	June 30	1.75				
	June 30	1.92				
1.13	June 29	1.92	2.08	0.32	1.05	2.36
	June 29	2.16				
	June 30	2.16				

Average value of constant in last column = 2.41. Maximum deviations = -3% .
 $+3\%$.

TABLE V.

$$\text{Formula: } \log T + k(\log C + 1) = K.$$

Potassium salts.

Salt.	k .	K .
Potassium iodide	2.09	0.94
Potassium nitrate	1.77	1.75
Potassium chloride	1.72	1.87
Potassium dichromate	0.67	0.23

Sodium salts.

Sodium iodide	1.38	0.75
Sodium nitrate	1.91	2.41
Sodium chloride	2.3	1.31

DISCUSSION OF RESULTS.

In each case graphs between $\log T$ and $\log C+1$ have been made and the points found to lie approximately upon straight lines. From the intercepts of these lines upon the axes the values of ' k ' have been found.

In the last column of Table IV the value of ' k ' has been substituted in the equation for each salt and the value of the second constant K calculated. The ranges of deviation have been shown.

It will be seen that the time range was limited as it was not possible to run the thermostat through the night. In a few cases, where long-period experiments were made, a tendency was found for the change in the

protoplasm to take place earlier than the calculated time. This tendency had already been found in experiments in dilute solutions at room temperature, but it took place with more concentrated solutions at 25° C. than was the case at the lower temperature. The equations shown are therefore only suggested as applying for a time range of ten minutes to four hours at this temperature. Table V shows the constants collected for the series of potassium salts and for that of sodium salts. It will be seen that ' k ' varies from 0.67 to 2.3, while K lies between 0.23 and 2.41. It is possible that the percentage error could have been reduced by still more care in selecting material so that there was greater uniformity in thickness of the cell walls, as this must have been a factor influencing the diffusion. It is interesting to examine the constants in the similar equation already found by other workers. In the work of Harvey (3) upon *Chlamydomonas* immersed in resorcin of various concentrations, the criterion used was cessation of movement. In this case ' k ' was 1.21, while K had a value between 3.75 and 3.58.

In the paper of Watson (2) the figures obtained by Chick for disinfection of bacteria were examined. Here the arbitrary measure of change was loss of power to multiply. The liquids used were phenol, silver nitrate, and mercuric chloride. The co-efficient ' k ' had values from 0.86 to 5.5 for different substances, while the constant K fell between 3 and 6.8.

In the paper of Harvey it is stated that the equation in question is one obeyed in a chemical reaction where one molecule of one compound reacts with ' k ' molecules of another compound, which is present in great excess, when the concentration ' C ' is made to vary and T is the time for the reaction to reach completion in each case. In the results recorded here it is clear that equimolecular solutions of two salts with monovalent kations and similar anions produce different effects, while marked differences occur if the kation be kept constant and the anion varied, so that simple chemical action does not seem to explain the changes produced. Of recent years great interest has centred round the absorption of ions by colloidal matter and consequent precipitations owing to electrical effects. The proteins of protoplasm are known to fall into the colloidal category.

Precipitations of colloids by electrolytes show many complications. There is a tendency for increased adsorption as the valency increases, but valency alone is not enough to consider. Wilder Bancroft (5) suggests there is 'specific adsorption' so that the concentration of a given electrolyte necessary to neutralize the charge upon a given colloid depends upon the nature of both kation and anion. According to Hardy (6) when we deal with living matter, or with organic compounds with large molecules, the influence of the electrolyte is determined not only by the charge carried by the ion but by the volume of the ion also. Further, precipitation

experiments carried out in test-tubes give different values according to the speed with which the electrolyte is added and according to the amount of agitation.

No theory can at present be put forward to account for the formula obtained in the experiments described, but there are certain facts, set out below, which seem to indicate that the poisonous action of these strong solutions of salts is bound up with adsorption of ions and coagulation of colloids of the protoplasm.

I. The value of ' k ' for the potassium salts, beginning with the smallest, runs in the order

dichromate < chloride < nitrate < iodide,

while the order of precipitation values for potassium salts acting upon colloidal iron oxide is found by Weiser and Middleton (7) to be exactly the same.

II. When experiments were made with salts of aluminium it was found far weaker concentrations sufficed to produce the critical change in a given time than sufficed with either potassium or sodium salts.

III. Deviations from the equation occur with the more dilute solutions, comparatively greater action taking place than with stronger solutions; these deviations seem comparable with those found for dye taken up from baths of different strengths by colloidal matter (8).

When the constants for sodium salts are examined it is curious to find that the co-efficient values, using different anions, run in exactly the reverse way to those found for potassium. It is interesting to find that in experiments by Pauli on anion adsorption by albumin cases were found where, in HCl concentration of 0.01 N to 2 N, the order of anion adsorption was reversed (5). With a view to finding simpler time relationships for coagulation of the colloids by electric charges it seems helpful to study a case in which charges of one kind only are used, instead of using an electrolyte where one may have negative ions, positive ions, and undissociated molecules. At the present time experiments are being made upon the same plant material under the action of radium. When the latter is contained in a platinum tube the α -rays, carrying positive charges, are cut out, and the plant cells can be submitted to the action of the β -rays, which carry negative charges. An endeavour is being made to connect time of action needed to produce the change in permeability with the intensity of radiation. There may possibly be certain effects to be allowed for due to the very 'hard' X-rays which accompany the β -rays.

Further light perhaps may be thrown upon the action of the salts by carrying out experiments with one particular electrolyte over a range of temperatures and finding the changes in k and K . It may also prove helpful to carry out experiments expressing results in terms of electric conductivities of solutions instead of molecular concentrations.

SUMMARY.

1. Experiments upon changes produced in cells of *Saxifraga umbrosa* by immersion in certain electrolytes have been repeated and extended. Times of immersion necessary to produce a certain arbitrary change, viz. permeability to 0.1 per cent. $K_2Cr_2O_7$, have been found for different concentrations of various salts.

2. The change of permeability referred to in this and earlier work has been shown to be irreversible and to indicate damage to or 'poisoning' of the cell.

3. The importance of immersion experiments at a fixed temperature has been shown.

4. The equation $\log T + k (\log C + 1) = K$,

where T = time of immersion needed to produce the change,

C = concentration in gram-mols. per litre,

has been shown to hold in the cases examined for periods up to four hours for immersions at 25° C.

5. This equation obtained for vegetative cells of an angiosperm poisoned by immersion in solutions of sodium and potassium salts has been found to agree in form with that obtained by other workers for disinfection of bacteria and for poisoning of *Chlamydomonas* by other chemicals.

6. The constant K and the coefficient k depend upon the salt used, and equimolecular solutions do not produce equal effects. Evidence is put forward in favour of the view that the action of the strong solutions employed is connected with the adsorption of ions and consequent precipitation of colloidal matter.

7. The need for study of the action of one kind of charge alone is shown and the suggestion made that the investigation of the action of the β -rays of radium may throw light upon the course of events.

In conclusion, the writer wishes to express her sincere thanks to Professor W. Stiles for certain references, to Sir Archibald Reid, K.B.E., C.M.G., for his generous loan of a large amount of radium for the present experiments, and to Mr. F. J. Harlow, in whose department the work has been carried out, for his kindness in arranging for the necessary apparatus and for his invaluable advice and criticism throughout the experiments.

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NOTE.

A NEW METHOD OF PARAFFIN INFILTRATION.—In preparing material for the microtome a certain amount of difficulty is usually experienced in passing from absolute alcohol to paraffin. The ordinary method is to employ a clearing agent, such as xylol, chloroform, bergamot oil, or cedar-wood oil, which is miscible with both absolute alcohol and paraffin.

Unless, however, the transition from (1) the alcohol to the clearing agent, and (2) from this to the paraffin, is *very* gradual, satisfactory sections are not obtained.

A similar difficulty arises in the previous process of dehydration when effected by passage through a series of alcohols of increasing strengths, and good results are usually only obtained by using a series, the members of which differ not more than 5 per cent. to 10 per cent. one from another.

A much more satisfactory method, involving only one change, and one to be recommended to the worker who has more than one 'job' on at a time, is to employ glycerine. The material after fixing and washing is placed in 10 per cent. glycerine and left open to the air in a convenient place to concentrate. At the end of two or three days the water will have evaporated away, and the material is left in nearly pure glycerine, which is easily and quickly replaced by absolute alcohol. The process of dehydration has been accomplished without any sudden change from a lower to a higher concentration, and the loss of water has been a uniform process.

It occurred to the writer that a similar method might be devised for the infiltration process which, if successful, would lead to equally satisfactory results. What was wanted was a solution of paraffin wax, corresponding to the 10 per cent. glycerine, which could be made to evaporate slowly and finally result in pure paraffin. The following method has been used at Wisley with some success on such material as soft-rot of turnips and *Peronospora parasitica* on *Capsella* stems. Mr. F. T. Brooks, who tried the method, has informed the writer that excellent results were obtained with such material as anthers prepared for cytological work. The method is as follows:

A small quantity of paraffin is melted in the oven and then poured into a graduated glass bottle which has been kept warm. Twice the volume of xylol is added, and then three times the volume of absolute alcohol. The bottle thus contains a liquid (at the oven temperature) made up of one part by volume of paraffin wax, two parts of xylol, and three parts of absolute alcohol. The liquid solidifies at room temperature and must be kept in the oven. Material in absolute alcohol is warmed in the oven for a few hours and then transferred to a tube of the paraffin mixture and left tightly corked for twenty-four hours.

It must not be forgotten that only one-sixth of the volume is wax and that five-sixths will pass off on evaporation, so that a sufficient quantity of the mixture is needed to leave enough wax to cover the material after evaporation. After twenty-four hours the cork is removed, when the alcohol and xylol will evaporate, and the tube will gradually smell less and less of xylol. In forty-eight hours the process should be complete, and the material will then be in pure paraffin wax. The transition from absolute alcohol to paraffin has been effected in one step and at a uniform rate.

Probably, it will be found that a modification of the quantities is needed for different material, and that the xylol can be replaced by any other volatile clearing agent.

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